

# **AN INVESTIGATION INTO THE ROLES OF HISTAMINE RECEPTORS IN THE CONTROL OF HUMAN NASAL BLOCKAGE**

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degree of Doctor of Philosophy in the University of London

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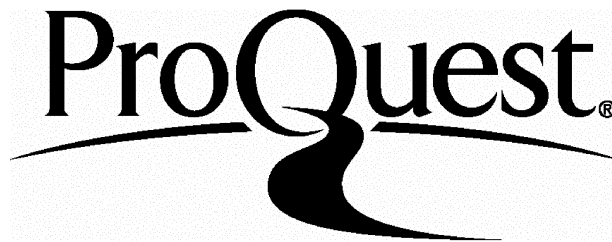
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## ABSTRACT

Allergic rhinitis is an allergic disease of the nose and, in sensitized individuals, is caused by inhaled innocuous particles such as pollen and house dust mite faeces. Allergen binds IgE on the surface of nasal mast cells, causing mast cell activation and degranulation, resulting in the release of inflammatory substances that are responsible for the symptoms of allergic rhinitis – sneezing, rhinorrhea, pruritus and nasal blockage. In this thesis, the mechanisms by which histamine, a mast cell-derived inflammatory mediator released during allergen challenge, causes nasal blockage were investigated. In addition, the role of nasal sympathetic neurones in the control of nasal blockage was also investigated.

The nasal blockage caused by inflammatory substances was assessed objectively by a technique called acoustic rhinometry. Subjects, either normal, healthy, individuals or atopic, allergic, individuals, were challenged with nasal aerosols of pollen, histamine or histamine receptor agonists, and their nasal responses were recorded. In this way, nasal blockage was shown to be caused by pollen, histamine, dimaprit ( $H_2$  receptor agonist) and R- $\alpha$ -methylhistamine ( $H_3$  receptor agonist). In addition, various histamine receptor antagonists were administered to the subjects to investigate to what extent these drugs affected responses to nasal challenge. Using this technique, histamine-induced nasal blockage was shown to be sensitive to  $H_1$ ,  $H_2$  and  $H_3$  antagonists and pollen-induced nasal blockage was shown to be insensitive to  $H_1$  and  $H_2$  antagonists.

Both noradrenaline and 3,4-dihydroxyphenylglycol (noradrenaline metabolite) were measured in nasal lavages. Pharmacological interference of the sympathetic nervous system led to functional changes in human nasal patency. In particular, antagonism of  $\alpha_1$ -adrenoceptors caused nasal blockage.

The presented data suggest that histamine causes nasal blockage via  $H_1$ ,  $H_2$  and  $H_3$  receptors. In addition, nasal sympathetic neurones were shown actively to maintain nasal patency and this was inhibited by activation of presynaptic  $H_3$  receptors.

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## PRESENTATIONS AND PUBLICATIONS

TAYLOR-CLARK TE (2001). Bradykinin and its role in seasonal allergic rhinitis. Awarded 2<sup>nd</sup> Prize GB West Memorial Essay Competition 2001.

TAYLOR-CLARK TE (2002). Possible role of the histamine H<sub>3</sub> receptor in human nasal blockage. XXXI Annual Meeting of the European Histamine Research Society, Eger, Hungary.

TAYLOR-CLARK TE, SODHA R, WARNER B, LIU WLS, PARRY J, FOREMAN JC (2004). A histamine H<sub>3</sub> receptor mediates blockage of the normal human nasal airway. *Br. J. Pharmacol.* Submitted for publication.

# ABBREVIATIONS

5-HETE	5-hydroxyeicosatetraenoic acid
5-HIAA	5-hydroxyindoleacetic acid
5-HTP	5-hydroxytryptophan
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ADH	Aldehyde dehydrogenase
AHR	Airway hyperreactivity
Amin.	Minimum cross-sectional area
ANOVA	Analysis of variance
Antag	Antagonist
AP-1	Activator protein-1
AR	Aldehyde reductase
ATP	Adenosine-5-triphosphate
AUC	Area under the curve
AVA	Arteriovenous anastomoses
BK	Bradykinin
Buf	Buffered saline
cAMP	Cyclic-3,5-adenosine-monophosphate
Cet	Cetirizine
CGRP	Calcitonin gene related peptide
Chlor	Chlorpheniramine
Clon	Clonidine
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
Cory	Corynanthine
COX	Cyclooxygenase
DHPG	3,4-dihydroxyphenylglycol
Dim	Dimaprit
DMA	3,4-dihydroxymandelic acid
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
ECP	Eosinophil chemotactic protein
EDHF	Endothelium dependent hyperpolarizing factor
EDRF	Endothelium-derived relaxing factor
EDTA	Ethylenediaminetetraacetic acid

EET	Epoxyeicosatrienoic acid
EPO	Eosinophil peroxidase
GABA	Gamma-amino butyric acid
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G protein coupled receptor
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HClO <sub>4</sub>	Perchloric acid
HDC	Histidine decarboxylase
Hist	Histamine
HMT	Histamine N-methyltransferase
HMWK	High molecular weight kininogen
HPLC	High pressure liquid chromatography
HPLC-ECD	Electrochemical high pressure liquid chromatography
HVA	Homovanillic acid
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IgA, IgE, IgG, IgM	Immunoglobulin A, Immunoglobulin E, Immunoglobulin G, Immunoglobulin M
IL-	Interleukin-
ILT	immunoglobulin-like transcript
Lid	Lidocaine
LIR	leukocyte immunoglobulin-like receptor
LMWK	Low molecular weight kininogen
L-NAME	N <sub>G</sub> -nitro-L-arginine methyl ester
L-NMMA	N <sub>G</sub> -monomethyl-L-arginine
LOD	Limit of detection
LTB <sub>4</sub> , LTC <sub>4</sub> , LTD <sub>4</sub> , LTE <sub>4</sub>	Leukotriene B <sub>4</sub> , Leukotriene C <sub>4</sub> , Leukotriene D <sub>4</sub> , Leukotriene E <sub>4</sub>
MAO	Monoamine oxidase
MBP	Major basic protein
MHC	Major histocompatibility complex
MHPG	3-methoxy, 4-hydroxyphenylglycol
MPO	Myeloperoxidase
NA	Noradrenaline
NAA	Noradrenaline aldehyde
N-acetyl-5-HT	N-acetyl-5-hydroxytryptamine
NFκB	Nuclear factor-κB
NKA	Neurokinin A

NM	Normetanephrine
NMA	Normetanephrine aldehyde
NO	Nitric oxide
NOS	Nitric oxide synthase
NPY	Neuropeptide Y
PAF	Platelet activating factor
PAR	Perennial allergic rhinitis
PGD <sub>2</sub> , PGI <sub>2</sub>	Prostaglandin D <sub>2</sub> , Prostacyclin
PKA, PKC, PKG	Protein kinase A, Protein kinase C, Protein kinase G
PLA <sub>2</sub>	Phospholipase A <sub>2</sub>
PNS	Peripheral nervous system
Ran	Ranitidine
RANTES	Released and normally T-cell expressed and secreted
ROI	Reactive oxygen intermediates
RαMeH	R-α-methylhistamine
Sal	Saline
SAR	Seasonal allergic rhinitis
SαMeH	S-α-methylhistamine
T <sub>H</sub>	T <sub>Helper</sub> lymphocytes
Thio	Thioperamide
TLR	Toll-like receptors
TNFα	Tumour necrosis factor α
U <sub>1</sub> , U <sub>2</sub>	Uptake <sub>1</sub> , Uptake <sub>2</sub>
VAS	Visual analog scores
VCAM-1	Vascular cell adhesion molecule 1
VIP	Vasoactive intestinal peptide
VLA-4	Very late antigen 4
VMA	Vanillylmandelic acid
VOCC	Voltage-operated Ca <sup>2+</sup> channels
Xylo	Xylometazoline
α <sub>2</sub>	α <sub>2</sub> -adrenoceptor
ΔAUC	Difference between two area under the curve values
ΔP	Pressure difference

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# CHAPTER 1

## INTRODUCTION

### 1.1 Allergic Rhinitis

Allergic rhinitis is defined as the abnormal inflammation of the lining of the nose provoked by an allergen, a substance which is harmless in normal individuals but causes inappropriate immune responses in sensitized individuals. Allergic rhinitis is characterized by nasal congestion, pruritus ("itchiness"), sneezing and rhinorrhea ("runny nose") as well as the development of airway hyperreactivity (AHR), which exacerbates any further allergic response. In addition to these symptoms, patients may also suffer from fatigue, irritability, sleep disturbance, headache and cognitive impairment (Nayak AS *et al.*, 2001). The number of people suffering from allergic rhinitis is difficult to gauge, as not all sufferers seek hospital or medical aid, however recent figures suggest that in the United States between 10-30% of adults and 40% of children are afflicted (Berger WE, 2003). The prevalence of allergic rhinitis appears to be increasing worldwide, particularly in industrial nations, thus the economic toll caused by allergic rhinitis is increasing: in the United States alone, the number of lost workdays resulting from allergic rhinitis is already estimated as being approximately 3.5 million a year (Holgate ST *et al.*, 2003).

Allergic rhinitis is an abnormal immune response to specific innocuous particles which have been trapped on the mucosal lining of the nose. These aeroallergens, usually sized from 5-70 $\mu$ m, include pollen from trees, grasses and weeds; and animal dander, whose sources range from rat urine to cat skin to the faeces of the house dust mite, *Dermatophagoids pteronyssinus*. The occurrence of symptoms for an individual depends on which aeroallergens he or she is sensitized to. Thus people who are allergic only to grass pollen will display symptoms only during the pollen season (typically for grasses between May and August), and these people are said to have seasonal allergic rhinitis (SAR). Those people who are allergic to house dust mite faeces will display symptoms all year round and are said to have perennial allergic rhinitis (PAR). There is increasing evidence to suggest that the difference between SAR and PAR is not just the duration of symptoms, but that the

pathophysiology of the two disease states may be different (Dear JW, 1996; van Wijk RG *et al.*, 1999).

Allergic rhinitis is not the only allergic disease. There are other allergic diseases which, although having similar pathogenesis and pathology, affect different areas of the body and are characterized by different symptoms: allergic asthma in the lower airways; systemic anaphylaxis; atopic dermatitis, angioedema and urticaria in the skin; and allergic conjunctivitis.

## 1.2 Structure and functions of the nose

The nose serves to warm and humidify inspired air whilst protecting the lungs from unwanted debris and pathogens. The nose is also the organ of olfaction.

An external bony frame surrounds the nasal cavity which is divided sagittally into two distinct parts by the cartilaginous septum. The nose protrudes from the face and has two apertures, called nostrils or nares, each one serving as the anterior opening to each side of the nasal cavity. Located posteriorly to each nostril is a nasal vestibule which contains stiff, bristly hairs which serve to trap large particles. Posterior to the nasal cavity, the two chambers combine to form the nasopharynx which leads caudally towards the larynx and the bronchial tree.

Three bony protrusions, called conchae or turbinates (inferior, middle and superior), on the lateral side of each chamber serve to increase massively the surface area of the nasal mucosa. The epithelium of the nasal mucosa is made up of ciliated and non-ciliated columnar cells and mucus goblet cells (Figure 1.1). The ciliated columnar cells gradually force the 10µm-thick layer of mucus (made by the mucosal goblet cells and the serous and mucus glands located in the submucosal layer) from the anterior of the chamber posteriorly. The mucus salt content is in equilibrium with interstitial fluid and contains glycoproteins, which increase mucus viscosity; and secretory Immunoglobulin (Ig) A, IgG, neutral endopeptidase, lactoferrin and lysozyme, all of which contribute to the anti-microbial environment (Lorin MI *et al.*, 1972; Thaete LG *et al.*, 1981).

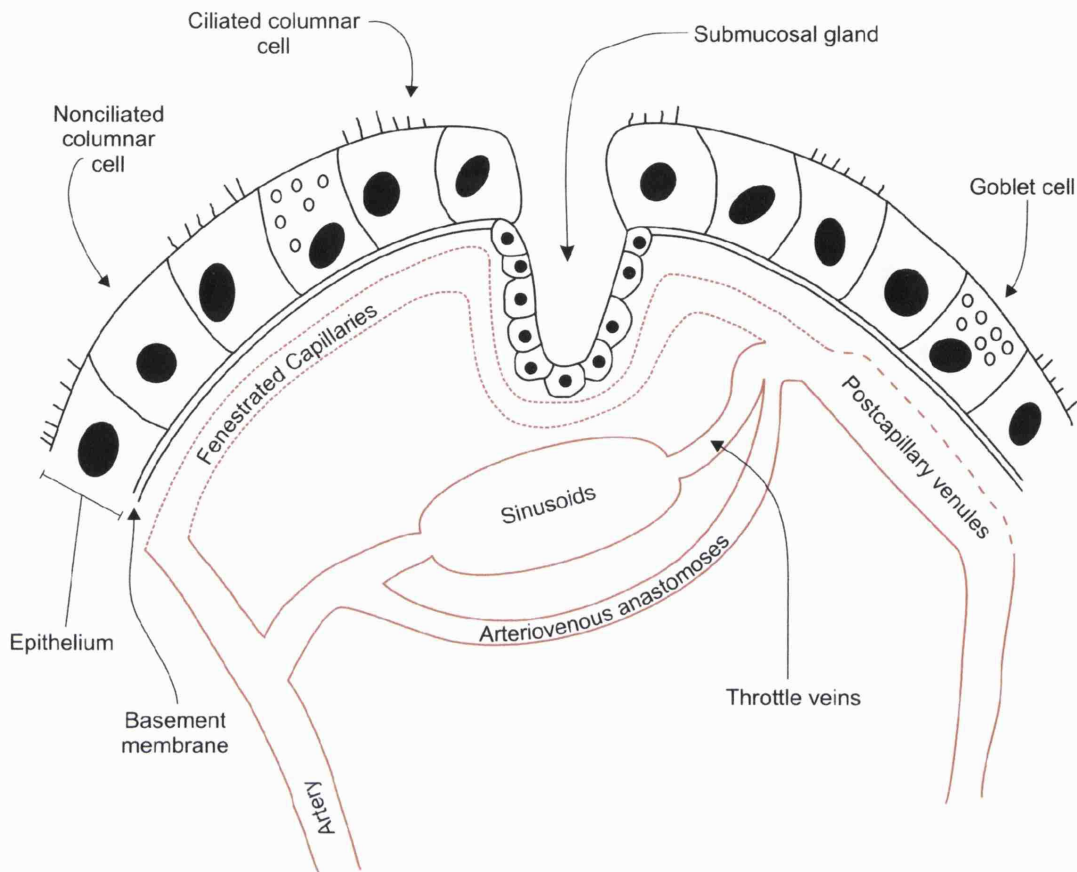


Figure 1.1: Diagram showing the cellular and vascular arrangement of the nasal mucosa.

Blood from the ophthalmic and internal maxillary arteries feeds into the huge network of arterioles, venules, capillaries, capacitance vessels and shunt vessels (Figure 1.1), which together supply the nasal mucosa with greater blood flow per volume of tissue than the liver or brain. The fenestrated capillaries, lying just beneath the epithelium, supply the glands and epithelium with oxygen and nutrients (Grevers G *et al.*, 1996).

The capacitance vessels, or sinusoids, are large cavernous blood vessels, situated beneath the capillaries in the submucosa, particularly in the turbinate tissue, which are able to fill with blood and expand (Dawes JD *et al.*, 1953). Since the nasal mucosa is surrounded by cartilage and bone, the expansion of the turbinate tissue causes protrusions into the airway, inhibiting airflow which results in a reduction in nasal patency (nasal blockage/congestion). The precise control of the sinusoidal engorgement is not clear. The vein-like capacitance vessels have unusually thick smooth muscle layers (Dawes JD *et al.*, 1953), which may be responsible for

contracting the blood vessel enough to prevent engorgement. Alternatively, the capacitance vessels may fill with blood or empty in a passive manner, under the control of other blood vessels. The capacitance vessels may fill with blood in response to dilation of the preceding arterioles, or in response to contraction of “throttle veins” situated directly after the sinusoids (Widdicombe J, 1997). It is possible that all three mechanisms occur during nasal blockage.

The role of the network of arteriovenous anastomoses (AVA) is also controversial. Some papers suggest that the AVA allow changes in blood flow through the mucosa without affecting airflow through the nasal cavity. Thus the AVA network serves as a temperature regulator of inspired and expired air (Atkinson TP *et al.*, 1995; Widdicombe J, 1997). The alternative viewpoint suggests that AVA feed into some capacitance vessels, thus AVA tone regulates sinusoidal engorgement (Dawes JD *et al.*, 1953; Anggard A, 1977; Stjorne P, 1991). The true answer to this controversy may lie in the accurate definition and identification of the vessels feeding the sinusoids. Alternatively, it may be explained by the fact that the majority of studies which showed a role of the AVA in the control of nasal patency were performed in animals, whereas the studies which showed no AVA control of nasal patency were performed in humans.

The postcapillary venules are fenestrated on the epithelial side and it is here that the majority of plasma extravasation occurs, replenishing the interstitial fluid which supplies the mucus exudate (Widdicombe J, 1997).

The nasal mucosa, including the glands and blood vessels, are supplied by both afferent and efferent neurones. The afferent neuronal supply can be divided into two parts: the first, the olfactory nerve (cranial nerve I), projects into the olfactory mucosa, which lies just above the middle turbinate, and conducts the sensation of smell; the second, cranial nerve V, projects to the epithelium and detects perception of airflow via A fibres, and noxious stimuli (both physical and chemical) via unmyelinated C fibres and A $\delta$  fibres. Activation of these afferent nerves leads to local axonal and central reflexes (Baraniuk JN, 1998). Although the nasal mucosa has no motor neurones, there is a substantial efferent neuronal supply in the form of the autonomic nervous system. Sympathetic neurones, branching from the superior cervical ganglion, innervate the smooth muscle of arterioles, AVA and capacitance

blood vessels; whilst parasympathetic neurones, via the sphenopalatine ganglion, innervate the submucosal glands and, to some extent, the surrounding blood vessels.

Thus the structure of the nose facilitates its specialized functions: the highly vascularised turbinates warm and humidify the inspired air, whilst the “mucus escalator” traps foreign debris and microbes as small as 10µm, and then transports them up the cavity towards the nasopharynx where they are swallowed.

### 1.3 Pathophysiology of allergic rhinitis

Allergic rhinitis is thought to be an excessive immune response to innocuous antigen, mediated by the products of mast cell activation and degranulation after the “innocent” allergen binds to IgE on the surface of the mucosal mast cells. However, it is important to note that there is evidence that some aeroallergens are biochemically active. Biochemical properties of allergens resemble those of hydrolytic enzymes, enzyme inhibitors, transport proteins, and proteases (Stewart GA *et al.*, 1993), and these properties may contribute to the IgE-mediated inflammatory environment or promote the initial sensitization of the individual to the specific antigen.

The pathophysiology of allergic rhinitis can be divided into two parts: the initial sensitization to the allergen, and the reactionary response to subsequent exposures.

#### 1.3.1 Sensitization

Allergen becomes trapped in or on the surface of the nasal mucosa. Epithelial dendritic cells and possibly macrophages, monocytes and epithelial cells, ingest the allergen, process it into short amino acid sequences that are then bound to antigen recognition sites on major histocompatibility complex (MHC) class II molecules. The dendritic cell migrates through the submucosa and presents the processed antigen to naïve undifferentiated T<sub>Helper</sub> (T<sub>H</sub>) lymphocytes. Antigen-specific T cells bind the dendritic cell MHC class II (with processed antigen) with CD4 and this interaction, along with other cell-cell signals, triggers the T cells to differentiate into T<sub>H2</sub> cells.

The stimulus for the allergen presentation by the dendritic cells is not completely understood. Protein allergen alone has been shown to be unable to activate dendritic cells *in vivo* and *in vitro*. It is thought that an additional signal, perhaps via the activation of Toll-like receptors (TLR) on dendritic cells by conserved microbial motifs, is able to facilitate this abnormal immune response to the allergen (Eisenbarth SC *et al.*, 2003). Once the dendritic cell is activated, why does it activate a  $T_{H2}$  response as opposed to the non-allergic  $T_{H1}$  response? The concept of two separate populations of dendritic cells, one which stimulates  $T_{H1}$  responses and one which stimulates  $T_{H2}$  responses, has been challenged by recent studies which show that dendritic cells are not pre-committed to either  $T_H$  responses but rather stimulate specific  $T_H$  activation in response to their environment (Edwards AD *et al.*, 2002). It is possible that dendritic cells stimulate  $T_{H2}$  responses due to the presence of an additional signal such as interleukin (IL) 4. Alternatively,  $T_{H2}$  responses may be activated if  $T_{H1}$ -associated signals, such as IL-12, are not present (Eisenbarth SC *et al.*, 2003).

In addition to dendritic cells phagocytosing allergen, allergen also cross-links membrane-bound antibodies on antigen-specific B cells, triggering B cell activation and allergen internalization. The activated antigen-specific B cell then presents processed antigen (bound to MHC class II) to the activated antigen-specific  $T_{H2}$  cell. This triggers a cascade of cytokine release (including IL-1, IL-2, IL-3, IL-4, IL-6, IL-13) and immune cell activation which results in the proliferation of the antigen-specific B and T cells, the development of memory cells and plasma effector cells, the inhibition of  $T_{H1}$  responses and the upregulation of IgE production from the antigen-specific B cells (Van Cauwenberge PB, 1997; Baraniuk JN, 1997).

IgE is a highly specialized antibody which, under normal circumstances, makes up a tiny percentage of total serum immunoglobulin content. Once heavy-chain switching to IgE has occurred following allergen sensitization, both serum and nasal IgE levels tend to rise, although symptoms of allergic rhinitis correlate better with nasal IgE (Marcucci F *et al.*, 2001). Circulating antigen-specific IgE binds (using the Fc region) to FcεRI receptors on the surface of nasal mast cells and basophils, exposing the antigen-specific Fab region to the local environment, ready to be activated by further allergen exposure.

The cause of the heavy-chain switch to IgE is not fully understood, but it is clear that without the production of IgE, the type 1 hypersensitivity of allergic rhinitis would not occur. Familial predisposition to allergy development suggests that genetic factors influence the IgE switch, with several polymorphisms in the genes encoding IL-4 and IL-13 having been linked with increased levels of IgE (Geha RS *et al.*, 2003). In addition, polymorphisms of genes encoding dendritic cell TLR have been linked to allergic asthma (Geha RS, 2003; Holgate ST *et al.*, 2003).

The increased prevalence of allergic rhinitis, particularly in the developed world, has led to the establishment of the “hygiene hypothesis”. The hypothesis argues that an environment devoid of viruses and bacteria reduces an individual’s development of  $T_{H1}$  cells, thus promoting  $T_{H2}$  pathways and allergy (Geha RS, 2003). Many studies have highlighted a link between exposure to microbes (such as in farming communities, or as a child with many older siblings) with reduced atopy (Holgate ST *et al.*, 2003; Geha RS *et al.*, 2003). There is particularly substantial evidence suggesting a negative relationship between infection with the common cold and allergic disease (Liu AH *et al.*, 2003). However, the “hygiene hypothesis” remains controversial as there is mounting conflicting evidence: for example, parasitic infection (which leads to  $T_{H2}$  responses) causes a decrease in atopy; and although exposure to mycobacteria and the measles virus reduces allergen sensitization, there is little evidence that these infections lead to a switch to  $T_{H1}$  responses or that they occur before the age at which adult patterns of immunological reactivity have already been established (Kemp A *et al.*, 2003).

### **1.3.2 Response to allergen after sensitization**

#### **1.3.2.1 Mast cell degranulation**

In an atopic, or sensitized, individual, the nasal mucosa is full of IgE-bound mast cells, all within the first 200µm of the mucosa. In addition, nasal biopsies in atopic individuals have shown a migration of mast cells through the submucosa to the epithelium (Enerback L *et al.*, 1986). Challenge with a specific aeroallergen will lead to allergen binding the IgE on the surface of these mast cells. Cross-linking of the IgE stimulates a signal transduction mechanism down through the  $Fc\epsilon RI$  which activates intracellular tyrosine kinases, such as Syk (Siraganian RP, 2003). Syk



activates phospholipase  $C_v$ , leading to the conversion of membrane phospholipid into inositol-1,4,5-trisphosphate and 1,2-diacylglycerol, which promote an increase in  $[Ca^{2+}]_{intracellular}$  and activate protein kinase C (PKC), respectively. Activation of these two pathways results in granule exocytosis (degranulation) and solubilisation of the granule contents. Mast cell granules contain pre-formed mediators and proteins, including histamine, platelet activating factor (PAF), eosinophil chemotactic protein (ECP), heparin, tryptase and, in about 15% of nasal mast cells, chymase (Malm L *et al.*, 1987).

Syk also activates a series of small GTPases, such as Rac and Ras. These GTPase cascades cause the activation of transcription factors and phospholipase  $A_2$  ( $PLA_2$ ) which lead to the production of cytokines and arachidonic acid, respectively. Mast cell activation causes the release of IL-4, IL-5, IL-6, IL-13 and tumour necrosis factor (TNF)  $\alpha$  (Malm L *et al.*, 1987; Howarth PH, 1995). Arachidonic acid is converted into prostaglandin  $D_2$  ( $PGD_2$ ), leukotrienes and 5-hydroxyeicosatetraenoic acid (5-HETE), which diffuse out across the plasma membrane (Mygind N, 1982).

Allergen-induced mast cell activation and degranulation causes an increase of inflammatory mediators and cytokines in the local environment which, together with the subsequent immune cell migration and activation, causes the symptoms of allergic rhinitis.

### 1.3.2.2 Cellular infiltration

Endothelial cells, stimulated by histamine and cytokines such as  $TNF\alpha$ , upregulate their expression of P-selectin and E-selectin on their luminal surface. These adhesion molecules bind oligosaccharide moieties on attracted eosinophils, neutrophils, basophils and T cells and induce rolling in these leukocytes. The activated leucocytes upregulate their expression of cell-surface ligands, such as  $\beta 2$ -integrin and very late antigen 4 (VLA-4), which bind to endothelial cell adhesion proteins (e.g. intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)), newly upregulated by IL-4, IL-5, IL-13 and  $TNF\alpha$  (Howarth PH *et al.*, 2000). E-selectin, ICAM-1 and VCAM-1 have all been shown to increase in nasal mucosal biopsies of atopic individuals following nasal allergen challenge

(Braunstahl GJ *et al.*, 2001). Interaction between leukocyte cell-surface ligands and endothelial cell adhesion proteins complete the tethering of the immune cells to the endothelium, and migration across into the interstitium then follows.

Epithelial cells are activated after allergen challenge and increase their expression of RANTES (released and normally T-cell expressed and secreted) and eotaxin. Both are chemokines which have been implicated in the recruitment of eosinophils, basophils and T cells into the epithelium (Howarth PH *et al.*, 2000). Activated epithelial cells express ICAM-1, which binds leukocyte integrins; and also release granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6 and IL-8, which maintain leukocyte survival and add to the chemotactic environment.

### **1.3.2.3 Role of immune cells in allergic rhinitis**

The inflammatory response after allergen challenge is a vastly complicated cascade of cell activation and mediator release, with a high degree of pleiotropism, synergy and redundancy. Activated leukocytes will, in turn, release cytokines which promote the migration of more cells into the inflamed area, thus it is difficult to arrange a satisfactory order to the immune cascade. Below is a description of the contributions made by the major players in the cellular recruitment which occurs during allergic rhinitis:

Eosinophils are granulocytes which, under normal conditions, account for a very small percentage of the body's leukocyte number. In allergic rhinitis, the number of eosinophils in the blood rises sharply and increased eosinophils have been found in nasal biopsies and nasal lavages in atopic individuals after allergen challenge (Miadonna A *et al.*, 1999; Nouri-Aria KT *et al.*, 2000; Braunstahl GJ *et al.*, 2001). Eosinophilic granules contain the toxins major basic protein (MBP), eosinophil peroxidase (EPO) and ECP, which cause desquamation and destruction of surrounding tissue *in vitro* (Motojima S *et al.*, 1989) in addition to activating basophils, neutrophils and eosinophils (Kita H *et al.*, 1995). Activated eosinophils release the contents of their granules after allergen challenge and MBP, EPO and ECP have all been measured in post-challenge nasal lavages (Nielsen LP *et al.*, 1998; Erjefalt JS *et al.*, 1999; Kovalhuk LC *et al.*, 2001). Eosinophils are also able to synthesize and release the chemokines eotaxin and IL-8 and the inflammatory

mediator leukotriene C<sub>4</sub> (LTC<sub>4</sub>). Eosinophils are attracted by IL-5, RANTES and eotaxin (Sampson AP, 2000) and are activated by histamine, PAF and T<sub>H2</sub> cytokines such as IL-3 and IL-5. Eosinophils also contain a histaminase which breaks down the mast cell-derived inflammatory mediator (Howarth PH, 1995). Eosinophils are thought to play a major role in the development of airway hyperreactivity (AHR) both in the lower (Costello RW *et al.*, 1999; Sampson AP, 2000) and upper (Turner PJ *et al.*, 1999) airways. However, AHR is able to develop without eosinophilia (Saito *et al.*, 2002).

The role of neutrophils, another type of granulocyte, in allergic rhinitis is more controversial. Neutrophils can be activated by histamine, PAF, GM-CSF, TNF $\alpha$ , IL-8 and leukotriene B<sub>4</sub> (LTB<sub>4</sub>). Increased neutrophils in nasal lavages after allergen challenge have been reported (Sampson AP, 2000), with the subsequent production of the toxins ECP (Sur S *et al.*, 1998) and myeloperoxidase (MPO); lipid mediators such as leukotrienes and PAF; and reactive oxygen intermediates (ROI). These products may contribute to nasal blockage and the development of AHR. However, ECP is also produced by eosinophils (Pronk-Admiraal CJ *et al.*, 2001), thus it is difficult to assess the contribution made by either cell type to ECP production. Additionally, corticosteroids, which are able to reduce AHR *in vivo*, reduce neutrophil apoptosis *in vitro* (Sampson AP, 2000). Several studies investigating topically administered steroids on allergic rhinitis show a reduction in symptoms and AHR, but no effect on the associated neutrophilia (Togias A *et al.*, 1988; Naclerio RM, 1990; Benson M *et al.*, 2000), although in another study steroids reduced both AHR and neutrophilia (Walden SM *et al.*, 1988).

Basophils are blood-borne cells, similar to mast cells, which have IgE bound, via Fc $\epsilon$ RI, to their plasma membrane. In allergic rhinitis the number of basophils in the nasal mucosa increases (Sampson AP, 2000), and they degranulate after coming into contact with allergen, resulting in similar increases in mediator release (histamine, leukotrienes), except for tryptase and PGD<sub>2</sub> which basophils are unable to release in any significant amount. Basophils are attracted by RANTES, IL-3, IL-5 and IL-8 (Baraniuk JN, 1997) and thought to play an important role in the late phase response to allergen.

CD4<sup>+</sup> T<sub>H2</sub> cells have been found to increase in the nasal mucosa in allergic rhinitis (Baraniuk JN, 1997). These lymphocytes are able to release IL-2, IL-3, IL-4, IL-5, IL-10, IL-13, GM-CSF and TNF $\alpha$  (Howarth PH, 1995), which reduce granulocyte apoptosis and maintain the T<sub>H</sub> balance in favour of T<sub>H2</sub>. After allergen challenge in atopic individuals, T<sub>H2</sub> cells are thought to promote the eosinophilia and neutrophilia in addition to stimulating B cells to produce IgE. T<sub>H2</sub> cells are attracted by RANTES, and activated by TNF $\alpha$ , IL-2 and IL-10.

Other cells types have also been shown to increase in allergic rhinitis: macrophages, dendritic cells, monocytes, B cells (Bachert C *et al.*, 1998); although their roles in the response to allergen challenge in less clear.

#### 1.3.2.4 Plasma-derived mediators

Nasal lavages performed after allergen challenge show increases in bradykinin, lys-bradykinin, plasma kallikrein and tissue kallikrein (Proud D *et al.*, 1983; Baumgarten CR *et al.*, 1986a; Baumgarten CR *et al.*, 1986b). Kinins are vasoactive inflammatory substances, capable of causing vasodilation and activation of sensory neurones and leukocytes. The trigger for kinin production is not clear. However, the kinin cascade is notoriously easy to activate: for example, kinins are released from blood when it comes into contact with glass (Margolis J, 1958).

Kinins are formed from the cleavage of inactive precursor kininogens (both high molecular weight kininogen (HMWK) and low molecular weight kininogen (LMWK)) by the activated enzyme kallikrein. There are two types of kallikrein, tissue and plasma, with the former able to release lys-bradykinin from both HMWK and LMWK; and the latter able to cleave HMWK into bradykinin and, in the presence of neutrophil elastase, cleave LMWK into lys-bradykinin. Tissue kallikrein can be activated by N-terminal proteolytic enzymes, such as trypsin, plasmin and plasma kallikrein (Proud D *et al.*, 1988a). Plasma kallikrein is activated by coagulation factor XII (Hageman factor), after the factor itself has been activated following exposure to negatively charged moieties, such as some basement membrane components and mast cell-derived heparin.

Given the ease of kinin formation, it is not surprising that kinin levels increase after allergen challenge. Activated granulocytes release proteolytic enzymes which can activate tissue kallikrein and expose interstitial membrane components. These negatively charged membrane components, as well as heparin (released after mast cell degranulation), could activate Hageman factor, which activates plasma kallikrein. In addition, it has been shown that mast cell-derived tryptase can also contribute to kinin formation. Tryptase can activate plasma kallikrein, but is also able to release kinin directly from kininogen, a process which appears to require neutrophil elastase (Imamura T *et al.*, 1996; Kozik A *et al.*, 1998).

The role of the complement and coagulation cascades in allergic rhinitis is not clearly understood. However, as mentioned above, factor XII of the coagulation cascade, or Hageman factor, is a stimulus for the nasal production of kinins. As allergic rhinitis is not mediated by activated IgG or IgM antibodies, any possible activation of complement cascade is likely to be via the alternative pathway. Both C3a and C5a have been shown, in some reports, to release histamine from mast cells and basophils (Hartman CT Jr. *et al.*, 1981; Schulman ES *et al.*, 1988; Gerard NP *et al.*, 2002). In addition, C5a has been shown to have direct effects on the vasculature: causing vasodilation (Luo HY *et al.*, 1995) and an increase in vascular permeability (Jose PJ *et al.*, 1981).

### 1.3.2.5 Vascular events after allergen challenge

Allergen challenge results in the release of many vasoactive pro-inflammatory substances from mast cells, leukocytes, plasma and neurones; including histamine, PGD<sub>2</sub>, LTB<sub>4</sub>, LTC<sub>4</sub>, leukotriene D<sub>4</sub> (LTD<sub>4</sub>), leukotriene E<sub>4</sub> (LTE<sub>4</sub>), bradykinin, substance P, calcitonin gene-related peptide (CGRP), neurokinin A (NKA), vasoactive intestinal peptide (VIP), and acetylcholine (ACh) (Anggard A, 1977; Naclerio RM *et al.*, 1983; Togias A *et al.*, 1988; Widdicombe JG, 1990). In addition, some receptors for these vasoactive mediators have also been shown to be present on the nasal vasculature: histamine receptors are located on capacitance vessels; leukotriene receptors are found on capacitance vessels; VIP, CGRP and NKA receptors are present on arterial vessels, while substance P receptors are found on arterial, venous and capacitance vessels; and muscarinic (M<sub>3</sub>) receptors are

present on arterial and capacitance vessels (Baraniuk JN, 1992; Kaliner MA, 1994; Simons FE *et al.*, 1996; Shirasaki H *et al.*, 2002).

The nasal mucosa also contains substances which reduce inflammation, namely noradrenaline and neuropeptide Y. Both are released from sympathetic neurones and activate receptors on the nasal vasculature (Mygind N, 1982; Baraniuk JN *et al.*, 1992).

Allergen challenge results in increased nasal blood flow in both humans (Rangi SP *et al.*, 1990b) and pigs (Alving K *et al.*, 1990). Blood flow increases are a result of vasodilation, either by direct action on the smooth muscle or indirectly via activation of the vascular endothelium. Direct vasodilation, for example by PGD<sub>2</sub>, results from the activation, via sarcolemma G protein coupled receptor (GPCR) pathways, of protein kinase A (PKA) and protein kinase G (PKG). PKA and PKG reduce free  $[Ca^{2+}]_{intracellular}$ , which prevents myosin phosphorylation and leads to relaxation of vascular smooth muscle (Walsh MP, 1994). Indirect or endothelium-dependent vasodilation is a two stage process: first a vasoactive substance, such as bradykinin, substance P or histamine, activates a GPCR on the endothelial cell, which results in the release from the endothelium of a diffusible intermediate, such as nitric oxide (NO), prostacyclin (PGI<sub>2</sub>) or endothelium-dependent hyperpolarizing factor (EDHF). The intermediate then diffuses to the vascular smooth muscle cell where it activates the PKA and PKG pathways, lowers free  $[Ca^{2+}]_{intracellular}$ , again resulting in smooth muscle relaxation and vasodilation.

Vasodilation of different blood vessels results in different nasal symptoms. Vasodilation of the capillaries and postcapillary venules would increase the blood flow to the epithelium, potentially increasing the pressure for capillary plasma exudation. Vasodilation of the AVA, in man, would only lead to a rise in mucosa temperature (Widdicombe J, 1997). Vasodilation of the capacitance vessels or the arterioles which fed them, would lead to sinusoidal engorgement and the subsequent enlargement of the nasal mucosa. The enlarged turbinate tissue protrudes into the airways, obstructing airflow and causing the sensation of congestion (Cole P *et al.*, 1983). Allergen-induced nasal blockage, measured subjectively and objectively, has been reported on numerous occasions (Corrado

OJ *et al.*, 1987; Holmberg K *et al.*, 1989c; Johnston SL *et al.*, 1993; Baroody FM *et al.*, 1994; Zweiman B *et al.*, 1997).

Vasoactive substances such as histamine and bradykinin are also able to increase the vascular permeability of blood vessels (Killackey JJ *et al.*, 1986; Paul W *et al.*, 1994), an effect also seen after nasal allergen challenge (Baroody FM *et al.*, 1994; Paul W *et al.*, 1994). The bradykinin-, histamine- and allergen-induced increase in nasal vascular permeability has been shown to be reduced by inhibitors of NO synthase (the enzyme responsible for the production of NO), suggesting that the process is, in part, endothelium-dependent (Dear JW *et al.*, 1996a). An *in vitro* study of cultured endothelial monolayers showed that together with an increase in permeability, histamine caused disruption of cell junctions leading to the creation of gaps through which solutes could pass (Killackey JJ *et al.*, 1986). Another study reported the possibility of the reversible formation of gap junctions between endothelial cells in guinea-pig cutaneous capillaries in response to bradykinin and histamine (Paul W *et al.*, 1994). The increased vascular permeability is particularly marked in postcapillary venules where the opening of the intercellular gaps, together with the anatomical fenestrations, provides the plasma with an alternative route other than along the blood vessel. The plasma seeps into the interstitial space and is then forced through the epithelium into the nasal cavity as exudate (Widdicombe J, 1997). This process of mediator-induced vascular permeability is no doubt potentiated by any increase in dilation of the preceding blood vessels, thus increasing the pressure on the exudate to escape into the lumen of the nasal cavity. The exudate caused by increased vascular permeability is almost unfiltered plasma, containing albumin, antibodies and complement fractions (Bousquet J *et al.*, 1996). The exudate does not contain, to any great degree, lactoferrin or lysozyme, which are markers for glandular secretion (Raphael GD *et al.*, 1991).

The increase in vascular permeability and vasodilation which occurs during allergic rhinitis appears to be mediated locally without the influence of central reflexes. The majority of reports in the literature show that unilateral challenge with allergen or histamine show little or no contralateral changes in nasal patency (Kirkegaard J *et al.*, 1983; Baroody FM *et al.*, 1994). However, one study showed a decrease in contralateral blood flow following nasal allergen challenge, which was sensitive to the local anaesthetic lidocaine, possibly indicating a central reflex leading to an

increase in sympathetic activity followed by vasoconstriction (Holmberg K *et al.*, 1989a). In addition, another study, using histamine nasal challenge, showed a decrease in nasal resistance in the contralateral side (Birchall MA *et al.*, 1993). However, histamine has also been shown to *increase* nasal resistance in the contralateral side (Shelton D *et al.*, 1994).

#### 1.3.2.6 Neuronal events after allergen challenge

Small diameter C fibres innervate nasal blood vessels, submucosal glands and the epithelium (Baraniuk JN, 1992). Inflammatory mediators released during allergen challenge are able to sensitize (by inhibiting neuronal after-hyperpolarization) or directly activate (through increasing PKC phosphorylation of neuronal ion channels, which results in depolarisation and action potential initiation) the nerve endings. Neuronal excitation can also be increased by two other factors: firstly, cytotoxic proteins such as MBP and ECP may disrupt the mucosa, exposing the nerve endings to greater insults (both chemical and physical); secondly, cytokines such as IL-1 $\beta$  and TNF $\alpha$  can increase the expression of receptors on the neuronal membranes, e.g. increase in bradykinin B<sub>1</sub> receptor in allergic rhinitis (Christiansen SC *et al.*, 2002).

Activation of nasal afferents results in local reflexes, central reflexes and sensation. Neuropeptides, including substance P, CGRP and NKA, are released from neurosecretory swellings on afferent neurones following depolarization (Baraniuk JN, 1998). These neuropeptides contribute to the inflammatory milieu by causing vasodilation, an increase in vascular permeability and glandular activation (Baraniuk JN, 1997). The depolarization propagates up the neurone, which reaches up through the trigeminal sensory root in the central nervous system (CNS) and terminates in the pars caudalis of the nucleus of the spinal tract in the lower medulla and upper three cervical segments of the spinal cord. Interneurones from the pars caudalis innervate the thalamus via the trigeminothalamic tract, where the sensation of pain and itch is experienced, and tertiary neuronal networks stretch to the parietal cortex to provide spatial localization of the stimuli.

The trigeminal spinal tract is connected to the tractus solitarius and the nucleus ambiguus. The central reflexes induced by allergic rhinitis are mediated through



these interneurons. One reflex is the stimulation of motoneurons which control sneezing. Sneezing serves to quickly expel inhaled particles. Sneezing occurs rapidly after allergen challenge, but also only lasts a few minutes, suggesting the sneeze reflex is susceptible to tachyphylaxis. Another important reflex is the stimulation of nasal parasympathetic neurones which results in increased glandular activation.

#### **1.3.2.7 Glandular events after allergen challenge**

Allergen challenge results in increased glandular secretion, mainly via the indirect parasympathetically-mediated reflex (Widdicombe JG, 1990), although substance P is thought to directly stimulate the nasal mucosal glands (Barnes PJ, 1990). ACh, released from the parasympathetic neurones, activates M<sub>1</sub> and M<sub>3</sub> receptors on epithelial cells and submucosal glands causing the release of mucus (which contains lactoferrin, lysozyme, neutral endopeptidase, secretory IgA and glycoproteins) (Okayama M *et al.*, 1993; Baraniuk JN, 1998). Glandular secretion and plasma extravasation combine to cause the rhinorrhea associated with allergic rhinitis.

#### **1.3.2.8 Immediate/Late phase responses**

One peculiar characteristic of allergic rhinitis is the time course of symptom expression after a single allergen challenge. The immediate phase starts almost instantaneously with a short bout of sneezing, coupled with the rapid development of congestion, rhinorrhea and pruritus which last for 30 minutes. Approximately 4-12 hours after nasal challenge, symptoms re-appear (in particular nasal blockage) in what is termed the late phase response. This phenomenon does not occur in every atopic individual but appears to correlate with a rise in inflammatory mediator production (e.g. histamine, kinins, leukotrienes) in the nasal mucosa, as individuals without late-phase symptoms produce no mediator release during that time (Walden SM *et al.*, 1988). The reasons behind the late phase response are not clear. Although the late phase appears to be IgE related (Naclerio RM *et al.*, 1985; Kobayashi M *et al.*, 2001), there is evidence which suggests that the inflammatory mediator release is mediated by basophils and not mast cells. Firstly, unlike the immediate reaction after allergen challenge, the late phase does not lead to the

formation of PGD<sub>2</sub> or tryptase (Naclerio RM *et al.*, 1985; Zweiman B *et al.*, 1997) and basophils are unable to produce these mediators in large quantities. Secondly, after unilateral allergen challenge, histamine is only released into the ipsilateral nasal cavity; whereas in the late phase, histamine can be recovered in both nasal cavities, suggesting the involvement of a blood-borne histamine-releasing cell (Naclerio RM, 1997). Finally, basophils have been shown to increase in the nasal mucosa during the late-phase response (Naclerio RM, 1990).

### 1.3.2.9 Airway Hyperreactivity (AHR)

Another hallmark characteristic of allergic rhinitis is the development of AHR. AHR is a reversible increased nasal response to nonspecific inflammatory mediators, such as histamine, bradykinin, methacholine, tobacco smoke and perfume (Baraniuk JN, 1997; van Wijk RG *et al.*, 1999). AHR exacerbates the symptoms of allergic rhinitis. AHR develops within approximately 4 to 6 hours and can last for 24 hours. Although the time periods of the late phase response and the development of AHR are similar, it has been shown that a late phase response is not required for the development of AHR in the nasal airways (Togias A *et al.*, 1988; van Wijk RG *et al.*, 1992).

The mechanisms behind the development of AHR are not well understood, although potential pathways have been postulated: increased exposure of receptors to inflammatory mediators (due to damage and destruction of epithelial and interstitial cells by cytotoxic proteins, such as MBP and ECP); increased receptor expression; modulation of ligand-receptor affinity and efficacy; an increase in the half-life of inflammatory substances (due to a reduction in their metabolism); increased neuronal sensitivity; altered intracellular signaling; and increased mediator release (Mygind N, 1982; van Wijk RG *et al.*, 1999).

The development of AHR can also be induced by other substances: PAF in man (Austin CE *et al.*, 1993); N<sub>g</sub>-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, in man (Turner PJ *et al.*, 2000b); nerve growth factor in guinea-pigs (de Vries A *et al.*, 2001); and MBP in rats (Coyle AJ *et al.*, 1993). Although these studies highlight potential candidates for the development of AHR after allergen

challenge, so far efforts to identify the direct link between allergen and AHR have been in vain.

## **1.4 Therapeutic targets for allergic rhinitis**

Although many inflammatory mediators are released, and many immune cells are activated in allergic rhinitis, the contribution made by some of them to the symptoms of the disease is often slight. The problem faced by researchers is the redundancy, synergy and pleiotropism which exists amongst the mediators of the disease. Another problem is the lack of adequate experimental tools, such as selective receptor antagonists, although this should be a temporary hindrance.

### **1.4.1 Anti-IgE and mast cell stabilizers**

A monoclonal antibody, omalizumab, which targets IgE, has been shown to be effective in reducing symptoms associated with both seasonal and perennial allergic rhinitis (Casale TB, 2001; Chervinsky P *et al.*, 2003). Omalizumab binds to circulating IgE at the IgE domain that binds onto mast cell FcεRI receptors (Holgate ST *et al.*, 2003), thus preventing IgE binding to nasal mast cells. Omalizumab treatment results in a decrease (up to 90%) in IgE serum levels. An added beneficial effect is the internalization of mast cell FcεRI receptors which occurs if IgE does not bind them. However, monoclonal antibodies are subject to metabolism, thus patients require subcutaneous injections every three or four weeks. In addition, whilst omalizumab can reduce serum IgE levels by up to 90%, one study showed no symptomatic relief despite a reduction in serum IgE of 50% (Holgate ST *et al.*, 2003). This result shows that almost total abolition of serum IgE is required for any clinical efficacy to be shown. Induction of native anti-IgE antibodies has not been attempted in man but animal models have shown it to be efficacious in reducing IgE levels long-term (Yang P *et al.*, 1995).

Mast cell stabilizers, such as theophylline and disodium cromoglycate have been shown to be effective in reducing immediate phase symptoms in allergic rhinitis. Their mechanism of action is not clearly understood, but they reduce the levels of inflammatory mediators in the upper airways after allergen challenge and are

thought to prevent exocytosis of mast cell granules (Kunkel G *et al.*, 1987; Naclerio RM, 1990; Bousquet J *et al.*, 1996).

Mast cells possess inhibitory receptors on their plasma membranes, including immunoglobulin-like transcripts (ILT) and leukocyte immunoglobulin-like receptors (LIR) which, upon activation, reduce IgE-dependent degranulation by interacting with Fc $\epsilon$ RI receptors (Holgate ST *et al.*, 2003). ILT and LIR represent novel targets for reducing allergen-induced inflammatory mediator release.

#### 1.4.2 Steroids

Glucocorticoids (steroids) are used successfully to suppress inflammation in chronic inflammatory diseases, such as asthma and allergic rhinitis. Steroids are very potent substances with wide-ranging anti-inflammatory effects. In addition to these anti-inflammatory effects, steroids can alter salt-water homeostasis, alter protein and carbohydrate metabolism and reduce immunity to infection. Thus steroid treatment, particularly if systemically administered, is limited by potentially serious side-effects (Schimmer BP *et al.*, 1996).

Glucocorticoids bind the glucocorticoid receptor (GR) in the cytosol of most cells. The glucocorticoid displaces bound heat shock protein from the GR and binds to the GR, causing a conformational change that allows the glucocorticoid-GR complex to dimerise and migrate into the nucleus of the cell. The activated GR is then able to alter gene transcription in the cell nucleus by a number of direct and indirect mechanisms (Barnes PJ, 1998). The activated GR can bind to gene promoter sites called glucocorticoid response elements (GRE) and alter the rate of transcription. Lipocortin, the PLA<sub>2</sub> inhibitor, is upregulated in this way after steroid treatment, resulting in the inhibition of arachidonic acid production (thus reducing prostaglandin and leukotriene synthesis). Alternatively, GR can prevent the binding of pro-inflammatory transcription factors (e.g. activator protein-1 (AP-1) and nuclear factor- $\kappa$ B (NF $\kappa$ B)) to DNA, either directly e.g. binding of AP-1 by GR; or indirectly, by upregulating the gene transcription of I $\kappa$ B which then binds and inhibits NF $\kappa$ B. Another mechanism by which glucocorticoids alter gene transcription is through modulation of chromatic structure. Deacetylation of histones causes tighter coiling of the DNA, thereby preventing the access of pro-inflammatory transcription factors

to their promoter sites. Through these mechanisms, steroids are able to inhibit the upregulation of cytokines and adhesion molecules, and suppress the actions of immune cells during an allergic response.

Oral steroids are able to reduce eosinophilia, late phase mediators and symptoms and the development of nasal AHR (Togias A *et al.*, 1988). Nasal steroids, in addition to the effects caused by oral steroids, are able to reduce immediate phase mediators and symptoms (including nasal blockage), neutrophilia, and the release of TNF $\alpha$ , IL-1, IL-3, IL-5, IL-6, IL-8, IL-13 and RANTES (Walden SM *et al.*, 1988; Sim TC *et al.*, 1995; Kleinjan A *et al.*, 2000).

Steroids are potent vasoconstrictors (Kornel L, 1993). Although the mechanism by which this occurs is not fully understood, it is thought to be a direct effect on vascular smooth muscle. Steroid-induced vasoconstriction occurs immediately and is short-lived, thus it is unlikely that this mechanism contributes to symptom alleviation as steroids require days of treatment before effective symptom relief. Due to this therapeutic lag, steroid treatment is best taken prophylactically.

### 1.4.3 Antihistamines

Although inhibition of L-histidine decarboxylase (enzyme responsible for histamine production) has no effect on nasal symptoms caused by allergen (Pipkom U *et al.*, 1987), antihistamines have been used as a mainstay treatment for allergic rhinitis for nearly fifty years. Both anaphylaxis and allergy were understood to be mediated, in part, by histamine (Dragstedt CA *et al.*, 1932; Feldberg W, 1953), later shown to be acting at histamine 1 (H<sub>1</sub>) receptors (Ash AS *et al.*, 1966; Casterline CL *et al.*, 1977). The first generation of H<sub>1</sub> antagonists (e.g. chlorpheniramine, pyrilamine and diphenhydramine) were able to cross the blood brain barrier and, at clinical doses, caused sedation, diminished alertness and slowed reactions. Second generation H<sub>1</sub> antagonists (e.g. cetirizine, loratadine and terfenadine) have now been developed which are unable to cross the blood brain barrier. Antagonism of the H<sub>1</sub> receptor, by either first or second generation antihistamines, reduces allergen-induced sneezing, itching and rhinorrhea (Holmberg K *et al.*, 1989c; DuBuske L, 1995), although the effect of H<sub>1</sub> antagonists on allergen-induced nasal blockage is controversial (See Chapter 1.6.3). Antagonism of histamine 2 (H<sub>2</sub>) receptors does not appear to be

efficacious in the treatment of allergic rhinitis and H<sub>2</sub> antagonists are not used clinically for this purpose (Holmberg K *et al.*, 1989c).

#### 1.4.4 Anticholinergics

Muscarinic antagonists, such as atropine and ipratropium, are only able to reduce allergen-induced rhinorrhea (Barody FM *et al.*, 1994; Kaiser HB *et al.*, 1995), due to their inhibition of the parasympathetic-mediated glandular activation.

#### 1.4.5 Sympathomimetics

Drugs which activate  $\alpha$ -adrenoceptors are effective, both topically and systemically, in reducing allergen-induced nasal blockage (Johnson DA *et al.*, 1993; Hochban W *et al.*, 1999), because of vasoconstriction of the nasal vasculature, particularly the arterioles and capacitance vessels. Overuse of sympathomimetics is not recommended as it can lead to necrosis of the nasal mucosa and also may cause a paradoxical rebound congestion after treatment cessation (Naclerio R *et al.*, 1997).

#### 1.4.6 Leukotriene antagonists

Although exogenous LTC<sub>4</sub> and LTD<sub>4</sub> both produce nasal blockage when administered nasally (Miadonna A *et al.*, 1987), inhibitors of 5-lipoxygenase (enzyme responsible for leukotriene production) have shown varying results in treating the symptoms of allergic rhinitis (Knapp HR *et al.*, 1994). Nevertheless, the 5-lipoxygenase inhibitor, zileuton, has been shown to reduce nasal blockage and leukotriene levels after nasal allergen challenge (Knapp HR, 1990). Evidence supporting leukotriene antagonists, such as montelukast and zafirlukast, as efficacious in the treatment of allergic rhinitis is also equivocal (Maynard ML *et al.*, 2001). Montelukast was shown to be effective in reducing seasonal allergic rhinitis symptoms scores over a four week period (Van Adelsberg J *et al.*, 2003). However, another trial showed that montelukast over the course of the entire grass pollen season was only effective in reducing symptom scores when in combination with an H<sub>1</sub> antagonist (Pullerits T *et al.*, 2002). In addition, zafirlukast was shown to be effective in reducing allergen-induced symptom scores but this effect was not dose-related, with the highest dose being ineffective (Busse WW, 1996).

### 1.4.7 Platelet activating factor (PAF)

PAF attracted much attention as a target for allergic rhinitis after it was shown to cause eosinophilia and the development of AHR. It is also able to increase the release of kinins and ECP and, at higher doses, causes nasal blockage (Austin CE *et al.*, 1993; Turner PJ *et al.*, 2000a). PAF antagonists, however, have not yet progressed from animal model testing, where they appear to reduce some effects of allergen-induced symptoms (Albert DH *et al.*, 1998).

### 1.4.8 Bradykinin antagonists and neuropeptide antagonists

Bradykinin antagonists, such as icatibant (also known as Hoe-140), are able to reduce both allergen-induced and PAF-induced nasal AHR and eosinophilia (Turner PJ *et al.*, 2000a; Turner *et al.*, 2001). Bradykinin, however, fails to produce AHR when administered nasally. Inhibition of bradykinin metabolism, using angiotensin converting enzyme (ACE) inhibitors, potentiates ozone-induced AHR in guinea-pig lung *in vivo* (Matsubara S *et al.*, 1997), although other studies have shown no effect of ACE inhibitors on the development of AHR in the lower airways of man (Overlack A *et al.*, 1992; Diczpinigaitis PV *et al.*, 1996). No studies into the effects of ACE inhibitors on nasal AHR have been published, although ACE inhibitors have been shown to potentiate bradykinin nasal responses in atopic individuals (Proud D *et al.*, 1990; Gotoh M, 1999). Nasal bradykinin is also broken down by carboxypeptidase N (Proud D *et al.*, 1987), and perhaps inhibition of this enzyme may lead to AHR.

Bradykinin antagonists are able to abolish the nasal blockage caused during PAR, but not SAR (Austin CE *et al.*, 1994b), indicating that the pathologies of SAR and PAR are indeed different and that bradykinin is responsible for nasal blockage in PAR.

Neuropeptides have been linked with the development of AHR. Systemic capsaicin, which reduces neuropeptide release by preventing C fibre activation and depleting neuropeptide stores, inhibits allergen-induced AHR in the lower airways of male Wistar rats; whereas an inhibitor of neutral endopeptidase (enzyme that metabolizes neuropeptides) increased the AHR in the lower airways of normal rats (Chiba Y *et al.*, 1994). In addition, an antagonist at the substance P receptor, NK<sub>1</sub>,

reduced the hyperreactivity caused by nerve growth factor in guinea-pig tracheal rings *in vitro* (de Vries A *et al.*, 2001). Finally, exogenous substance P caused the development of AHR in the lower airways of guinea-pigs *in vivo* (Evans CM *et al.*, 2000). These studies show that neuropeptides, such as substance P, NKA and CGRP, play a role in the development of AHR in rats and guinea-pigs. However, substance P is unable to cause AHR in the lower airways in man (Cheung D *et al.*, 1994) and an antagonist at the NKA receptor, NK<sub>2</sub>, does not inhibit the development of bronchial AHR in atopic individuals after allergen challenge (Kraan J *et al.*, 2001). Nevertheless, repeated nasal administration of NKA to human subjects caused nasal AHR and an increase in eosinophils in nasal lavages (Turner PJ, 1999). In the same study, substance P produced a similar response to NKA but the effect was not significant.

The role of neuropeptides in the immediate phase response is difficult to gauge as there is a lack of adequate pharmacological tools; particularly selective antagonists safe for human studies. When applied nasally to man, substance P causes nasal blockage and plasma extravasation (Devillier P *et al.*, 1988; Braunstein G *et al.*, 1991; Fajac I *et al.*, 1995; Konno A *et al.*, 1996), although another human study could not elicit any effects from nasally administered substance P (Miadonna A *et al.*, 1988). Exogenous NKA applied nasally causes only plasma extravasation (Braunstein G *et al.*, 1991), and CGRP causes nasal blockage (Rangi SP *et al.*, 1990a; Chatelain C *et al.*, 1995). Despite these effects of exogenous neuropeptides, local anaesthetics, which prevent the propagation of action potentials in C fibres and thus the release of neuropeptides, have no effect on allergen-induced symptoms, indicating neuropeptides may not play a crucial role in symptom generation (Holmberg K *et al.*, 1989b).

#### **1.4.9 Eosinophils, cytokines and adhesion molecules**

The eosinophil has long been regarded as the major cell involved in allergic rhinitis after the mast cell. Eosinophils have especially been implicated with the development of nasal AHR in humans (Durham SR *et al.*, 1985; Turner *et al.*, 2001), thought to be, in part, due to the release of destructive toxins such as MBP and ECP. In guinea-pig lung, eosinophil-derived MBP causes the development of AHR (Evans CM *et al.*, 1997). However, human studies using anti-IL-5 monoclonal



antibodies have shown a reduction in blood and tissue eosinophilia but no effect on lung AHR (Saito *et al.*, 2002). In addition, there is no correlation between eosinophilia or ECP levels and nasal AHR (van Wijk RG *et al.*, 1999). Eosinophils and their products may not be responsible for AHR in man.

IL-5 is not the only cytokine to be investigated as a possible target for the treatment of allergic rhinitis. Unfortunately, to date, there are no published studies of cytokine antagonists in humans with allergic rhinitis. However, animal studies and cytokine challenges in humans help illustrate their effect in nasal allergy. IL-10 knock-out mice have a reduced incidence of tracheal AHR compared to wild-type (Justice JP *et al.*, 2001). Inhibition of IL-13 reduces nasal AHR in allergen-challenged mice (Holgate ST *et al.*, 2003). Nasally applied IL-8 causes nasal blockage in man (Douglass JA *et al.*, 1994), and neutrophilia and the development of AHR in guinea-pig lung (Lu K *et al.*, 1997). IL-4 causes nasal blockage in man, but also reduces the sensitivity of the nasal mucosa to histamine challenge (Emery BE *et al.*, 1992).

Chemokines and adhesion molecules are upregulated in allergic rhinitis (Gangur V *et al.*, 2003; Lloyd CM *et al.*, 2003). Researchers are beginning to focus on these molecules as possible treatment targets. P-selectin-deficient mice have been shown to have a reduced eosinophilia. Inhibition of VLA-4 reduces lung AHR in some animal models, although human clinical trials have failed to show this effect (Holgate ST *et al.*, 2003). Clinical trials of anti-eotaxin monoclonal antibodies and CCR3 (eosinophilic eotaxin receptor) antagonists in allergic rhinitis are in progress. It remains to be seen whether or not the redundancy and synergy of cytokines and chemokines renders any attempt of single cytokine/chemokine inhibition as inefficacious, regardless of the importance of that substance in allergic rhinitis.

#### **1.4.10 Nitric oxide synthase (NOS) inhibitors**

Nitric oxide (NO) production is increased in allergic rhinitis, with all three isoforms of NOS present in the nasal mucosa. NO can cause vasodilation, glandular secretion and is also a free radical (Baraniuk JN, 1997). The NO donor, sodium nitroprusside, caused nasal blockage after nasal administration to normal individuals (Imada M *et al.*, 2002). NOS inhibitors have been shown to reduce nasal blockage in PAR but not SAR (Dear JW *et al.*, 1996a), probably due to their ability to reduce NO-

dependent bradykinin-induced nasal blockage, and bradykinin has been shown to be responsible for nasal blockage caused by perennial allergens (Austin CE *et al.*, 1994b). NOS inhibitors are also able to reduce the plasma extravasation in SAR (PAR is not characterized by a significant increase in plasma extravasation) (Dear JW *et al.*, 1996a). In addition, knock-out mice deficient in inducible NOS have reduced cell infiltration, vascular leakage and nasal blockage after allergen challenge (Xiong Y *et al.*, 1999). However, NOS inhibitors, when nasally administered to human subjects, caused the development of upper airways hyperreactivity and significant eosinophilia (Turner PJ *et al.*, 2000b).

#### **1.4.11 Cyclooxygenase (COX) inhibitors**

Although PGD<sub>2</sub> increases after allergen challenge, and prostacyclin is thought to be involved in endothelium-dependent vasodilation, inhibitors of COX (enzyme responsible for prostaglandin production) fail to effect allergen-induced symptoms (Brooks CD *et al.*, 1984; Walden SM *et al.*, 1988).

#### **1.4.12 Heparin**

Heparin is thought to be able to reduce the development of AHR, an ability unrelated to its anticoagulant effects (Tyrrell DJ *et al.*, 1999). Heparin has been shown to reduce allergen-induced eosinophilia in human nasal airways *in vivo* (Crimi N *et al.*, 1995; Vancheri C *et al.*, 2001) and also reduces human leukocyte adhesion *in vitro* (Lever R *et al.*, 2000; Smailbegovic A *et al.*, 2001). Heparin is also able to bind to and inhibit the actions of toxic proteins such as MBP, ECP and EPO (Tyrrell DJ *et al.*, 1999). Heparin has been shown to reduce bronchial AHR in sheep (Ahmed T *et al.*, 1994) and in rabbits (Preuss JM *et al.*, 2000), but evidence for its efficacy in human lung is equivocal (Tyrrell DJ *et al.*, 1995; Tyrrell DJ *et al.*, 1999). No studies investigating the effect of heparin on human nasal AHR have yet been published.

#### **1.4.13 Immunotherapy**

Some studies have found that allergic symptoms are reduced after a prophylactic course of specific allergen challenges (Canonica GW *et al.*, 2003; Holgate ST *et al.*,

2003). The mechanism for this symptom amelioration is not clearly understood, but appears to involve a switch from a  $T_{H2}$  response to a  $T_{H1}$  response (Greenberger PA, 2002). A model of allergy and immunotherapy using bee venom and human T cell clones has shown that the concentration of allergen determines which  $T_H$  response is activated (Akdis CA *et al.*, 2000): lower levels of allergen induced a  $T_{H2}$  response with an increase in IL-4, whereas higher doses lead to a  $T_{H1}$  response and increased levels of interferon (IFN)  $\gamma$ . This switch has been reported in the clinic, where a reduction in allergen-induced nasal eosinophilia, AHR and symptoms has been achieved (Klimek L *et al.*, 1999) as well as an increase in cells expressing mRNA for IFN $\gamma$  (Durham SR *et al.*, 1998). Immunotherapy has also been shown to reduce T cell proliferation and ICAM-1 expression (Canonica GW *et al.*, 2003). However, the effect of immunotherapy on immunoglobulin levels differs from study to study (Canonica GW *et al.*, 2003): IgE levels have been shown to fall (Klimek L *et al.*, 1999) and increase (Akdis CA *et al.*, 2000) following treatment. Levels of IgG, an antibody which does not activate allergic mechanisms, have been shown to rise after immunotherapy (Durham SR *et al.*, 1998; Akdis CA *et al.*, 2000). Immunotherapy may also promote CD8 $^{+}$  T cells (Durham SR *et al.*, 1998; Akdis CA *et al.*, 2000), which suppress the development of atopy (de Sousa Mucida D *et al.*, 2003).

Benefit from immunotherapy often requires months of expensive regimented treatment. In addition, there is little by way of standardization of treatment, and results do vary (Canonica GW *et al.*, 2003). Treatment efficacy does not appear to be dose-related, although very high doses of allergen are rarely used because of resulting side-effects. Subcutaneous allergen injection has led to a number of fatalities, thus other routes of administration are being investigated; for example, sublingual (Naclerio R, 1997).

## **1.5 Techniques for investigating allergic rhinitis**

### **1.5.1 Nasal challenge**

In order to evaluate the roles played by inflammatory mediators and cells in allergic rhinitis, it is necessary to trigger the response by administering allergen to the nasal mucosa. Allergen tends to be administered via a solution or via paper discs (placed

onto the inferior turbinate). Allergen solutions can be instilled into the nose with a pipette or can be sprayed into the nose as an aerosol using a pump spray.

Most studies on seasonal allergic rhinitis occur outside the particular allergen's season. In this way the investigator can better control the exposure of the allergen to the experimental subjects. This unfortunately is not possible for studies into perennial allergic rhinitis and so the study design must take this "uncontrollable" nature of allergen exposure into account. In addition, it is thought that the clinical presentation of allergic rhinitis is not completely mimicked in single-challenge studies. Atopic individuals are continuously exposed to allergen outside the clinic – they are unlikely to get the short, sharp shock that a 100µl spray of concentrated allergen will give them. In this respect, studies that focus on the immediate phase response may have limited relevance to the true disease.

However, if single-challenge studies are seen as models for the clinical disease, they still represent a valuable tool for the investigation of allergic rhinitis, free from the inconsistencies of the late-phase response and the complication of AHR. The use of single-challenge studies is further justified by reports of good reproducibility for responses to allergen (Corrado OJ *et al.*, 1987; Doyle WJ *et al.*, 1995).

Allergen challenge causes, as described earlier, a series of humoral and cellular cascades which create a vastly complicated pathophysiological response. Exogenous mediator challenge can be a useful tool in piecing together the actions of a particular substance without the presence of other inflammatory mediators. Good reproducibility has also been shown for these mediator-challenges, including histamine (Corrado OJ *et al.*, 1987) and bradykinin (Churchill L *et al.*, 1991).

### 1.5.2 Functional measurements

Assessment of an individual's atopic status is a key prerequisite before their inclusion in any study. A case history can be taken which focuses on the appearance of obvious symptoms (sneezing, nasal congestion, runny nose) during specific times of year, or during specific activities (picnicking, gardening, dusting). A skin-prick test confirms the specific antigen which an individual is allergic to. In the test, spots of allergen solutions (plus a saline negative control and a histamine

positive control) are placed on the forearm and the skin under each spot is then pierced with a small needle. The subsequent wheals produced by the different allergens denote the specific allergens that the individual is sensitive to.

#### 1.5.2.1 Nasal blood flow and nasal blockage

The degree of vasodilation in the capacitance vessels and pre-capacitance arterioles determines the expansion of the turbinate tissue into the nasal cavity. Increased turbinate size results in nasal blockage and the sensation of nasal congestion. Laser Doppler flowmetry and  $^{133}\text{Xe}$  washout techniques can be used to measure the blood flow in the nasal mucosa (Olsson P, 1986), although blood flow in the mucosa does not always correlate with a change in airflow through the cavity (Lung MA *et al.*, 1984; Holmberg K *et al.*, 1989c; Wight RG *et al.*, 1989; Holmberg K *et al.*, 1990). This may result from the recording of increased blood flow in the AVA network which, in man, is thought not to contribute to the control of nasal patency (Widdicombe J, 1997). Objective measurements of nasal blockage fall into two categories: those that measure airflow and the pressure through the airways; and those that measure the patency or geometry of the nose.

#### 1.5.2.2 Airflow / Pressure measurements

Assessing nasal peak flow is a cheap, quick and easy method to quantify the airflow through the nose. Both inspiratory and expiratory measurements are possible, but the inspiratory technique is preferred as it does not clog up the flow meter with mucus as the expiratory technique can (Malm L, 1997). Nasal peak flow measures the airflow from the lungs through the nose to the outside air, and does not identify unilateral nasal changes or changes in the lung.

Rhinomanometry is a common technique for measuring the pressure difference between the nostrils and the buccal cavity. Results from rhinomanometry studies have shown good reproducibility and a coefficient of variance of approximately 10% (Pastorello EA *et al.*, 1994). The pressure difference ( $\Delta P$ ) relates to airflow through the nasal airways as described by Ohm's law:

$$\Delta P = I.R$$

Where  $I$  represents the airflow and  $R$  is the nasal resistance caused by the nasal cavity.

Rhinomanometry measurements are quick but require a degree of subject training. In addition, airflow is required for any measurement to be quantified, thus complete nasal blockage (with no airflow) cannot be recorded (Austin CE *et al.*, 1994c).

### 1.5.2.3 Patency / geometric measurements

Changes in turbinate size after nasal challenge can be shown using computer tomography (Cole P *et al.*, 1983), however this technique requires expensive machinery and subjects must maintain a stationary position for 20 minutes, making it unsuitable for most studies.

Acoustic rhinometry uses acoustic reflection to map out the contours of the nasal cavity (Hilberg O *et al.*, 1989). Acoustic rhinometry calculates both the cross-sectional area along the nasal cavity and its volume. Airflow through a tube is dependent on the point of most resistance, thus recording the minimum cross-section area ( $A_{min}$ ) is preferred to measuring the volume of the cavity (Austin CE *et al.*, 1994c). In addition,  $A_{min}$  recordings have been reported to correlate better with rhinomanometry measurements. If we assume that the nasal passage is a simple tube through which air flows without turbulence, the radius,  $r$ , is a function of the  $A_{min}$ :

$$A_{min} = \pi.r^2$$

The radius relates to the airflow through the tube as described by Poiseuille's law, which states:

$$I = \frac{\pi \Delta P . r^4}{8 \eta l}$$

Where  $I$  is the rate of airflow,  $\Delta P$  is the pressure difference across the airways,  $\eta$  is the viscosity of air and  $l$  is the length of the airway.

Using Ohm's law, airflow can be related to resistance through the airways:

$$R = \frac{\Delta P}{I}$$

Thus, by substitution, Poiseuille's law can be rewritten (assuming linear non-turbulent airflow):

$$R = \frac{8 \eta l}{\pi . r^4}$$

Therefore the resistance is inversely proportional to the radius to the fourth power. Subsequently, a small decrease in  $A_{min}$ . (and thus a small decrease in radius) will lead to a large increase in resistance to airflow.

Acoustic rhinometry is a quick method with virtually no subject-training needed. The coefficient of variance is low, approximately 7% (Fisher EW *et al.*, 1994), and airflow is not required thus rhinometry is suitable for even very congested subjects. Finally, each nostril is measured independently, so unilateral changes in nasal patency can be easily measured.

#### 1.5.2.4 Symptom scores

Nasal blockage can also be assessed subjectively by patients using symptom scores. These measuring systems vary from study to study but either the patient is asked to grade from 1 to 5 their congestion (with 1 denoting no congestion and 5

denoting fully congested) or the patient is presented with a chart with a horizontal line (the left side denoting no congestion and the right, fully congested) which they mark accordingly. Subjective scores of congestion should only be used as a secondary measure of nasal blockage. Symptom scores have a large coefficient of variation and there are reports of a lack of correlation between objective and subjective measurements of nasal blockage (Li X *et al.*, 1997). This is particularly evident after challenges with mediators which activate neuronal receptors, such as L-menthol which produces subjective relief from nasal blockage but with no actual change in airflow, nasal resistance or turbinate size (Eccles R *et al.*, 1990; Naito K *et al.*, 1997).

Symptom scores can also be used to assess other symptoms, particularly itch which would be hard to objectively quantify in man. Counting sneezes is a simple way of assessing the allergic response.

Although objective measurements of allergic rhinitis are preferable for the investigator, it must be remembered that any effective treatment must result in subject-experienced symptom relief. If a drug prevented nasal blockage but the subject still felt congested then there would not be much of a market for that treatment.

### **1.5.3 Nasal lavages, biopsies and assays**

Washing out the nasal cavity with saline (lavage) is a very useful method of assessing the humoral and cellular activities in allergic rhinitis. Assays for proteins, such as albumin or lactoferrin, and other substances, such as histamine, kinins and prostaglandins, give an indication of the degree of plasma extravasation, glandular activation and mediator release at given time points. Cells such as eosinophils and neutrophils can be separated and counted using staining and microscopy.

Use of nasal lavages assumes that the level of a certain mediator in the collected lavage is proportional to the level of that mediator in the nasal mucosa. It also assumes that this proportionality is the same for each mediator and that it does not change in response to allergen challenge or drug treatment. These assumptions are



hampered by the metabolism and venous clearance of mediators (Austin CE *et al.*, 1996).

The recovery of the saline is approximately 80% of the instilled volume, but it is not uncommon to recover far less of the instilled volume (particularly if the subjected has severe nasal blockage). The effect of this loss of volume on mediator recovery is not known; it may have a concentrating effect but also may lead to a decrease in recovery. In addition, the level of mediator recovery may be affected by the degree of plasma extravasation and glandular activation; it is conceivable that the recovery of a certain plasma mediator increases in the lavage not because of an increase in its production or release but because more plasma passes into the nasal cavity as exudate. To combat this, mediator levels can be compared to albumin levels which can be used as a marker for plasma exudation.

High levels of mediators are often found in the initial lavage of a subject on any given day, this is thought to be the result of a gradual build up of mediators prior to lavage (Naclerio RM *et al.*, 1983). Thus a series of lavages is often performed before attaining a basal pre-challenge measurement of mediator release. Investigations into late phase responses should take this gradual build up into account when measuring mediator release 4 or 6 hours after allergen challenge.

Nasal biopsies are another invaluable method to quantify the histology of the nasal mucosal (Enerback L *et al.*, 1986). Together with cells recovered from lavages, nasal biopsies can be analysed using immunohistochemistry, radioligand binding, polymerase chain reaction and other *in vitro* techniques (Raphael GD *et al.*, 1989; Dear JW *et al.*, 1996b; Iriyoshi N *et al.*, 1996).

## 1.6 Histamine

Histamine (2-[4-imidazolyl]-ethylamine) was first discovered by Sir Henry Dale in 1910, after its isolation from *Ergotinum dialysatum* (Dale HH *et al.*, 1910). It was shown to be a uterine stimulant and have a hypotensive effect. During the next 20 years, anaphylaxis was characterized as a result of an antigen-antibody reaction in sensitized tissue, and histamine was found to mimic this reaction both *in vitro* and *in vivo* (Dale HH, 1929; Dragstedt CA *et al.*, 1932). In 1952, histamine was found in

mast cells (Riley JF *et al.*, 1952) and then, in the late 1960's, histamine receptor heterogeneity was suggested (Ash AS *et al.*, 1966), with the subsequent discovery of selective H<sub>2</sub> antagonists a few years later (Black JW *et al.*, 1972).

Since its first discovery, histamine research has spread far and wide. The identification of three distinct receptors (H<sub>1</sub>, H<sub>2</sub>, and H<sub>3</sub>) using selective ligands has been followed by the cloning of the H<sub>4</sub> receptor from human cDNA libraries (Nakamura T *et al.*, 2000). Through these receptors, endogenous histamine has been shown to play a variety of distinct physiological and pathological roles in the body: roles which have been targeted in medical treatment. H<sub>1</sub> antagonists, first discovered by Daniel Bovet, for which he later won a Nobel Prize, remain at the frontline of pharmacological treatment for allergic diseases like allergic rhinitis and urticaria; and Sir James Black won a Nobel Prize for his work on H<sub>2</sub> antagonists, which are used to treat duodenal ulcers. In addition, histamine has been identified as a regulator of immune cells and as a CNS neurotransmitter.

In this thesis, the role that histamine plays in the development of the immediate symptoms of allergic rhinitis, especially the symptom of nasal blockage, was investigated. Histamine, when nasally administered, mimics the immediate phase symptoms associated with allergic rhinitis: nasal blockage, rhinorrhea, sneezing and nasal itch. These symptoms develop rapidly, and then fade within 15-20 minutes after the local histamine concentration drops due to metabolism and venous clearance. It is important to note, however, that histamine has been shown to have effects on immune cells which have a longer onset and duration of action. In general, activation of H<sub>1</sub> receptors on eosinophils and neutrophils promotes chemotaxis which is reduced by H<sub>2</sub> receptor activation. In addition, H<sub>1</sub> receptor activation stimulates antigen-presentation and T<sub>H1</sub> responses, while H<sub>2</sub> receptor activation promotes T<sub>H2</sub> populations and IgE production (Akdis CA *et al.*, 2003). The role of H<sub>3</sub> and H<sub>4</sub> receptors is less clearly understood, although H<sub>3</sub> have been found on monocytes, dendritic cells and macrophages and H<sub>4</sub> have been found on mast cells, eosinophils, neutrophils and T cells (Schneider E *et al.*, 2002; Buckland *et al.*, 2003; Holgate ST *et al.*, 2003). H<sub>3</sub> and H<sub>4</sub> receptors are thought to modulate the production of cytokines in the lung (Sirois J *et al.*, 2000; Akdis CA *et al.*, 2003).

### 1.6.1 Histamine receptors and ligands

To date, four distinct receptors have been discovered: H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub>. All four are plasma membrane proteins coupled to various G proteins. Histamine is a full agonist at all the receptors. Below is a table showing some of the agonists and antagonists at the histamine receptors (van der Goot H *et al.*, 2000):

	H <sub>1</sub>	H <sub>2</sub>	H <sub>3</sub> *	H <sub>4</sub> *
Agonist	2-methylhistamine betahistidine	dimaprit impromidine	R-α-methylhistamine <sup>#&amp;</sup>	
Antagonist	cetirizine chlorpheniramine mepyramine	cimetidine famotidine ranitidine	thioperamide	

Table 1.1: Selectivity of histamine receptor ligands. \* Most ligands at H<sub>3</sub> receptors are comparably effective at H<sub>4</sub> receptors; with the exception of clobenpropit, which is an agonist at H<sub>4</sub> receptors and an antagonist at H<sub>3</sub> receptors (Buckland *et al.*, 2003). # The stereoisomer of R-α-methylhistamine, S-α-methylhistamine, is also an agonist at the H<sub>3</sub> receptor, but is 120 times less potent than the R isomer (Arrang JM *et al.*, 1987). & R-α-methylhistamine is several hundred times less potent at H<sub>4</sub> receptors than at H<sub>3</sub> receptors (Schneider E *et al.*, 2002).

### 1.6.2 Histamine is an inflammatory mediator

Histamine is synthesized from L-histidine by histidine decarboxylase (HDC). Mast cells show high HDC activity, HDC mRNA has been found in nasal turbinate tissue and its levels are higher in atopics (Hirata N *et al.*, 1999b). HDC activity has also been shown in dendritic cells and T cells, and is regulated by various cytokines such as IL-1, IL-3, IL-12, IL-18, GM-CSF and TNFα (Akdis CA *et al.*, 2003). Histamine is metabolized by histamine N-methyltransferase (HMT) into N-methylhistamine, which is then metabolized into N-methylimidazole acetic acid before urinary excretion. HMT mRNA has been found in nasal turbinate tissue and its levels are lower in atopics (Hirata N *et al.*, 1999b).

Nasal biopsies have shown mast cells (filled with secretory granules) in the nasal mucosa and electron microscopy has revealed ultrastructural evidence of

degranulation following allergen challenge (Enerback L *et al.*, 1986; Kaliner MA, 1994; Howarth PH, 1995). Allergen-induced degranulation of nasal mast cells leads to a rise in histamine recovered in nasal lavages both in the immediate and late phase of the allergic response (Naclerio RM *et al.*, 1983; Naclerio RM *et al.*, 1985; Miadonna A *et al.*, 1987; Walden SM *et al.*, 1988).

Mast cell degranulation can also be stimulated by molecules other than specific antigen. Substances such as compound 48/80, substance P and bradykinin are thought to activate mast cell degranulation via a non-IgE related mechanism (Mousli M *et al.*, 1991). Non-IgE-mediated degranulation of mast cells has been shown in human skin *in vitro* (Lowman MA *et al.*, 1988), in cat nasal mucosa *in vivo* (McLeod RL *et al.*, 1999a) and rat peritoneum *in vivo* (Mousli M *et al.*, 1989; Zhao QE *et al.*, 1996). However, evidence supporting non-IgE-mediated degranulation of human nasal mast cells is less clear cut. Histologically, nasal mast cell populations are similar to those in human lungs and are distinct from those in human skin (Mita H *et al.*, 1993). Human airway mast cells have been shown not to respond to classic non-IgE degranulators (Ennis M, 1982; Gomez E *et al.*, 1987; Otsuka H *et al.*, 1995). Furthermore, nasal lavages showed no increases in histamine release in humans after nasal challenge of bradykinin (Proud D *et al.*, 1988b; Baumgarten CR *et al.*, 1997), or substance P (Fajac I *et al.*, 1995; Baumgarten CR *et al.*, 1996) or NKA (Braunstein G *et al.*, 1991). However, substance P has been shown by other groups to induce histamine release from human nasal mast cells *in vitro* and that the release is higher in mast cells collected from atopic individuals (Schierhorn K *et al.*, 1995; Hanf G *et al.*, 2000). Additionally, orally administered cetirizine (10mg) and terfenadine (60mg), both H<sub>1</sub> antagonists, reduced bradykinin-induced nasal blockage in humans, but, although bradykinin was shown to induce histamine release from human nasal mast cells *in vitro*, no increase in histamine was detected in lavages following bradykinin challenge (Austin CE *et al.*, 1996).

That non-IgE-mediated degranulation in human airways mast cells produces equivocal results could be explained by the observation of heterogeneity in these mast cell populations. Almost all human skin mast cells contain both tryptase and chymase and are thus categorised as T/C mast cells. T/C mast cells degranulate after exposure to compound 48/80. It is now thought that not all airway mast cells are T mast cells, which do not contain chymase and are unresponsive to compound

48/80, and a degree of the airway mast cell population are, in fact, T/C mast cells (Malm L *et al.*, 1987). Therefore, this mast cell heterogeneity of the airway mast cell populations could explain the few studies that have shown degranulation by non-IgE molecules.

As mentioned earlier, exogenous histamine, when applied to the human nasal mucosa in the form of a spray solution, causes sneezing, pain, pruritus, rhinorrhea and nasal blockage (measured by symptom scores, rhinomanometry and acoustic rhinometry) (Miadonna A *et al.*, 1987; Mullins RJ *et al.*, 1989; Doyle WJ *et al.*, 1990; Rajakulasingam K *et al.*, 1993; Austin CE *et al.*, 1994c; Birchall MA *et al.*, 1996). Betahistine, the H<sub>1</sub> agonist, caused sneezing, pruritus, rhinorrhea and nasal blockage (measured by rhinomanometry), while impromidine, the H<sub>2</sub> agonist only caused nasal blockage (Shelton D *et al.*, 1994). Unilateral histamine challenges have produced some conflicting reports in relation to the response of the contralateral cavity (ipsilateral nasal blockage was, however, consistent in all reports): in one study both histamine and impromidine caused a minor increase in nasal resistance (Shelton D *et al.*, 1994); another study showed no contralateral effect after histamine challenge (Kirkegaard J *et al.*, 1983); and a third study showed a histamine-induced decrease in nasal resistance in the contralateral side (Birchall MA *et al.*, 1993).

### **1.6.3 Effect of H<sub>1</sub> and H<sub>2</sub> antagonists on nasal inflammation**

The presence of histamine receptors in human nasal mucosa has been studied: H<sub>1</sub> receptors have been shown, using autoradiography, on vascular endothelium of inferior turbinates (Okayama M *et al.*, 1992); H<sub>1</sub> receptor mRNA has been shown in turbinates, with increased levels in atopic individuals (Iriyoshi N *et al.*, 1996); and H<sub>2</sub> receptor mRNA has been found on the epithelium, serous cells and mucus cells, with increased levels in atopic individuals (Hirata N *et al.*, 1999a).

The effect of various H<sub>1</sub> and H<sub>2</sub> antagonists on histamine-induced symptoms in man are presented below:

Site	Drug	Results	Reference
Nasal	Diphenhydramine (H <sub>1</sub> )	↓ Nasal blockage	(Havas TE <i>et al.</i> , 1986)
Nasal	Diphenhydramine (H <sub>1</sub> ) & Cimetidine (H <sub>2</sub> )	Antagonist combination ↓ nasal blockage more effectively than H <sub>1</sub> antagonist alone	(Havas TE <i>et al.</i> , 1986)
Nasal	Cetirizine (H <sub>1</sub> )	↓ plasma extravasation	(Wood-Baker R <i>et al.</i> , 1996)
Nasal	Cetirizine (H <sub>1</sub> ) & Ranitidine (H <sub>2</sub> )	Only ↓ nasal blockage when in combination	(Wood-Baker R <i>et al.</i> , 1996)
Nasal	Chlorpheniramine (H <sub>1</sub> )	↓ Sneezing, rhinorrhea & nasal blockage	(Mygind N <i>et al.</i> , 1983)
Nasal	Ranitidine (H <sub>2</sub> )	↓ Nasal blockage	(Mygind N <i>et al.</i> , 1983)
Nasal	Cetirizine (H <sub>1</sub> )	↓ Nasal blockage, sneezing	(Wang DY <i>et al.</i> , 2001)
Nasal	Ranitidine (H <sub>2</sub> )	No effect on rhinorrhea	(Mygind N <i>et al.</i> , 1982)
Nasal	Cetirizine (H <sub>1</sub> )	↓ Nasal blockage	(Hilberg O <i>et al.</i> , 1995)
Nasal	Chlorpheniramine (H <sub>1</sub> )	↓ Sneezing, rhinorrhea, pruritus & nasal blockage *	(Kirkegaard J <i>et al.</i> , 1983)
Nasal	Chlorpheniramine (H <sub>1</sub> )	↓ Sneezing, rhinorrhea, pruritus & nasal blockage	(Secher C <i>et al.</i> , 1982)
Nasal	Ranitidine (H <sub>2</sub> )	↓ Nasal blockage	(Secher C <i>et al.</i> , 1982)
Nasal	Chlorpheniramine (H <sub>1</sub> ) & Ranitidine (H <sub>2</sub> )	Antagonist combination more effective in ↓ nasal blockage than either antagonist alone	(Secher C <i>et al.</i> , 1982)
Skin	Chlorpheniramine (H <sub>1</sub> )	↓ itch	(Davies MG <i>et al.</i> , 1980)
Skin	Cetirizine (H <sub>1</sub> )	↓ wheal & flare by 80%	(Campoli-Richards DM <i>et al.</i> , 1990)

Table 1.2: Effect of H<sub>1</sub> and H<sub>2</sub> antagonists on histamine-induced inflammation. \* Unilateral histamine challenge: nasal spray of H<sub>1</sub> antagonist chlorpheniramine was only effective if

administered ipsilaterally. Note: Nasal blockage denotes objectively measured parameter, either by rhinomanometry, acoustic rhinometry or nasal peak flow.

H<sub>1</sub> receptors appear to mediate the histamine-induced nasal symptoms of nasal blockage, sneezing, rhinorrhea and pruritus. In the skin, H<sub>1</sub> receptors appear to mediate itch as well as the wheal and flare response. The inhibition of nasal symptoms appears to be a local effect as an H<sub>1</sub> antagonist spray was only effective if administered in the same nasal cavity as the histamine (Kirkegaard J *et al.*, 1983). It must be noted however that these studies did not attempt to show complete abolition of histamine-induced nasal blockage by H<sub>1</sub> antagonists, and so it is unclear whether or not the H<sub>1</sub> receptor is solely responsible for histamine-induced nasal blockage. This observation is supported by the results from H<sub>2</sub> antagonism of histamine-induced nasal symptoms. Although H<sub>2</sub> receptors do not appear to mediate sneezing, pruritus or rhinorrhea; there is some evidence to support a role of H<sub>2</sub> receptors in mediating histamine-induced nasal blockage. Whether or not H<sub>1</sub> and H<sub>2</sub> receptor pathways are the only mechanisms involved in histamine-induced nasal blockage is yet to be established.

Allergen challenge causes histamine release and exogenous histamine causes symptoms synonymous with allergic rhinitis. Histamine's role in allergen-induced symptoms in man can be elicited using various H<sub>1</sub> and H<sub>2</sub> antagonists. A summary of the relevant literature is presented below:

Allergen	Drug	Results	Reference
Seasonal	Cetirizine (H <sub>1</sub> )	↓ Sneezing, pruritus, rhinorrhea and nasal blockage (symptom score)	(Wang D <i>et al.</i> , 1996)
Seasonal	Levocabastine (H <sub>1</sub> )	↓ Symptom scores, but no effect on nasal blockage (nasal peak flow)	(Corren J <i>et al.</i> , 1999)
Seasonal	Cetirizine (H <sub>1</sub> )	↓ Sneezing, pruritus and rhinorrhea only	(Falliers CJ <i>et al.</i> , 1991)

Seasonal and Perennial	Cetirizine (H <sub>1</sub> )	↓ Sneezing, rhinorrhea and pruritus. Minor ↓ nasal blockage (symptom score)	(DuBuske L, 1995)
Seasonal	Fexofenadine (H <sub>1</sub> )	↓ Pruritus and nasal blockage (nasal peak flow)	(Wilson AM <i>et al.</i> , 2002)
Seasonal	Desloratadine (H <sub>1</sub> )	↓ Pruritus and nasal blockage (nasal peak flow)	(Wilson AM <i>et al.</i> , 2002)
Seasonal	Cetirizine (H <sub>1</sub> )	No effect on nasal blockage (acoustic rhinometry)	(Hilberg O <i>et al.</i> , 1995)
Seasonal and Perennial	H <sub>2</sub> antagonists	No effect on allergen-induced symptoms	(Atkinson TP <i>et al.</i> , 1995)
Seasonal	H <sub>1</sub> antagonists	↓ Sneezing and rhinorrhea only	(Naclerio RM, 1990)
Perennial	Desloratadine (H <sub>1</sub> )	↓ Sneezing, pruritus and rhinorrhea only	(Simons FE <i>et al.</i> , 2003)
Seasonal	Levocabastine (H <sub>1</sub> )	↓ Sneezing and rhinorrhea. No effect on nasal blockage (rhinomanometry)	(Holmberg K <i>et al.</i> , 1989c)
Seasonal	Ranitidine (H <sub>2</sub> )	↓ Rhinorrhea. No effect on nasal blockage (rhinomanometry)	(Holmberg K <i>et al.</i> , 1989c)
Seasonal	Levocabastine (H <sub>1</sub> ) & Ranitidine (H <sub>2</sub> )	Antagonist combination no effect on nasal blockage (rhinomanometry)	(Holmberg K <i>et al.</i> , 1989c)
Seasonal	Desloratadine (H <sub>1</sub> )	↓ Nasal blockage (peak flow)	(Horak F <i>et al.</i> , 2002)
Seasonal	Desloratadine (H <sub>1</sub> )	↓ Nasal blockage (symptom score)	(Nayak AS <i>et al.</i> , 2001)
Seasonal	Desloratadine (H <sub>1</sub> )	↓ Nasal blockage (symptom score)	(Schenkel E <i>et al.</i> , 2002)
Seasonal	H <sub>1</sub> antagonists	No effect on nasal blockage	(Vamey V, 1991)



Seasonal and Perennial	H <sub>1</sub> antagonists	No effect on nasal blockage	(Wood-Baker R <i>et al.</i> , 1996)
Seasonal	H <sub>1</sub> antagonists	No effect on nasal blockage	(Bousquet J <i>et al.</i> , 1990)
Perennial	Loratadine (H <sub>1</sub> )	↓ Sneezing, pruritus and nasal blockage (symptom score)	(Miadonna A <i>et al.</i> , 1998)
Seasonal	Terfenadine (H <sub>1</sub> )	↓ Nasal blood flow	(Juliussen S <i>et al.</i> , 1996)
Seasonal	Cimetidine (H <sub>2</sub> )	No effect on nasal blood flow	(Juliussen S <i>et al.</i> , 1996)

Figure 1.3: Effect of H<sub>1</sub> and H<sub>2</sub> antagonists on allergen-induced nasal symptoms.

The evidence suggests that histamine, via H<sub>1</sub> receptors, is to some degree responsible for the sneezing, pruritus and rhinorrhea caused after allergen challenge. Whether or not histamine is responsible for allergen-induced nasal blockage is more controversial. A review of the relevant literature suggests that H<sub>1</sub> antagonists in general are ineffective in reducing allergen-induced nasal blockage, although some studies show a decongestant effect by desloratadine. Decongestant effects may be revealed in other H<sub>1</sub> antagonists at higher doses. Alternatively, this decongestant effect by desloratadine may not result from antagonism at H<sub>1</sub> receptors. Receptor binding studies have shown that compared to the highly selective H<sub>1</sub> antagonists, cetirizine and fexofenadine, desloratadine is relatively unselective for H<sub>1</sub> receptors (Gillard M *et al.*, 2003): desloratadine bound to all five human muscarinic receptors, with K<sub>i</sub> values ranging from 20 to 50nM. The selectivity ratios between muscarinic receptors and H<sub>1</sub> receptors for desloratadine were approximately 50 to 125, compared to >20,000 for cetirizine. These binding studies suggest that the decongestant effect of desloratadine, unseen in other H<sub>1</sub> antagonists, may not result from antagonism of histamine at H<sub>1</sub> receptors but by some other mechanism: histamine-related or otherwise.

H<sub>2</sub> receptor mechanisms do not appear to mediate (to any significant degree) any of the allergen-induced symptoms, except, perhaps, rhinorrhea.

If histamine is responsible for the allergen-induced nasal blockage, it is possible that it is via mechanisms other than H<sub>1</sub> or H<sub>2</sub>.

### 1.6.4 H<sub>3</sub> receptor

The possibility of a distinct third histamine receptor was first considered following the investigation of histamine autoreceptors on histaminergic central neurones. Rat cortical slices pre-incubated with <sup>3</sup>H-histidine, depolarized by 30mM KCl, released <sup>3</sup>H-histamine, which was reduced by non-radioactive histamine (Arrang JM *et al.*, 1983). This histamine inhibition of <sup>3</sup>H-histamine release was unaffected by H<sub>1</sub> antagonists, although some H<sub>2</sub> ligands, such as the partial agonist impromidine and the antagonist burimamide, antagonized the presynaptic actions of histamine. Comparison of the affinity of these ligands for the presynaptic autoreceptor and for H<sub>2</sub> receptors showed that the autoreceptor had a different pharmacological profile to that of H<sub>2</sub> receptors.

In 1987, a paper presenting the first H<sub>3</sub> selective drugs, R- $\alpha$ -methylhistamine (agonist) and thioperamide (antagonist), was published (Arrang JM *et al.*, 1987). *In vitro* studies showed R- $\alpha$ -methylhistamine inhibited (<sup>3</sup>H) histamine release from depolarized rat cerebral cortex slices, whereas thioperamide shifted both the histamine- and R- $\alpha$ -methylhistamine-inhibition of (<sup>3</sup>H) histamine release dose-response curves to the right with an apparent K<sub>i</sub> of 1-10nM. R- $\alpha$ -methylhistamine had a relative potency at H<sub>3</sub> receptors of 1550% compared to histamine, whereas at H<sub>1</sub> and H<sub>2</sub> receptors its relative potency was 0.5% and 1%, respectively. Thioperamide also showed high selectivity for the H<sub>3</sub> receptor compared to other receptors such as H<sub>1</sub> (K<sub>i</sub>>100 $\mu$ M), H<sub>2</sub> (>10 $\mu$ M), adrenergic (>1 $\mu$ M) and muscarinic (>10 $\mu$ M) receptors. R- $\alpha$ -methylhistamine and thioperamide were then tested *in vivo* in rats injected with [<sup>3</sup>H] histamine, where they were shown to cross the blood brain barrier after intravenous administration. R- $\alpha$ -methylhistamine reduced [<sup>3</sup>H] histamine release from cerebral cortex, lung, abdominal skin and spleen, whilst thioperamide increased [<sup>3</sup>H] histamine release from cerebral cortex, hypothalamus and lung. In addition, radioligand binding studies using [<sup>3</sup>H] R- $\alpha$ -methylhistamine showed H<sub>3</sub> receptors throughout the rat brain, particularly in telencephalic areas, and also in guinea-pig lung parenchyma.

Since 1987, the genomic organization of the human H<sub>3</sub> receptor has been discovered (Coge F *et al.*, 2001), and the receptor cloned (Lovenberg TW *et al.*, 1999), with two splice variants being revealed (Tardivel-Lacombe J *et al.*, 2001). In

1991, H<sub>3</sub> receptors in rat cerebral cortex were shown to be G-protein coupled (Arrang JM *et al.*, 1991). Later, H<sub>3</sub> receptors responses on guinea-pig intestinal cholinergic neurones were shown to be mediated by G<sub>i/o</sub> (Blandizzi C *et al.*, 2001).

In humans, interest surrounding H<sub>3</sub> receptors has focused on those found in the CNS. H<sub>3</sub> receptors have been visualized in the basal ganglia and frontal cortex using autoradiography; and H<sub>3</sub> receptor mRNA has been found in the putamen, cortex and globus pallidus (Anichtchik OV *et al.*, 2001). Therapeutically, H<sub>3</sub> has been highlighted in the mechanisms involved in Alzheimer's and Parkinson's disease, appetite and sleeping disorders, epilepsy, and drug abuse (Arrang JM *et al.*, 1991; Anichtchik OV *et al.*, 2001; Alguacil LF *et al.*, 2003).

#### 1.6.4.1 H<sub>3</sub> is an inhibitory presynaptic receptor

The original work on H<sub>3</sub> receptors showed it to be an inhibitory autoreceptor on central histaminergic neurones (Arrang JM *et al.*, 1987). Inhibitory H<sub>3</sub> receptors have subsequently been found on both peripheral and central neurones containing virtually every type of neurotransmitter. Activation of H<sub>3</sub> receptors reduces noradrenaline (NA) release from renal sympathetic neurones in dogs *in vivo* (Yamasaki T *et al.*, 2001), from cardiac sympathetic neurones in guinea-pigs *in vitro* (Silver RB *et al.*, 2002), from mesenteric sympathetic neurones in guinea-pigs *in vitro* (Ishikawa S *et al.*, 1987), and from *in vitro* sympathetic neurones supplying the human saphenous vein (Molderings GJ *et al.*, 1992; Valentine AF *et al.*, 1999). It also reduces gamma-amino butyric acid (GABA) release from rat striatal neurones *in vitro* (Arias-Montano JA *et al.*, 2001); reduces ACh release from parasympathetic neurones in human bronchi *in vitro* (Ichinose M *et al.*, 1989), from intestinal cholinergic neurones in guinea-pigs *in vitro* (Blandizzi C *et al.*, 2001); and reduces histamine release from histaminergic neurones in rat hypothalamus *in vivo* (Jansen FP *et al.*, 1998).

Histamine binds the H<sub>3</sub> receptor on presynaptic membranes, activating pertussis-sensitive G<sub>i/o</sub>, reducing the depolarization of the presynaptic membrane and inhibiting the exocytosis of neurotransmitter vesicles. In guinea-pig intestinal neurones, the pertussis-sensitive H<sub>3</sub>-inhibition of ACh release is not affected by cyclic-3,5-adenosine-monophosphate (cAMP) analogues nor by inhibitors of PKA or

PKC. The  $H_3$ -inhibition of ACh release is enhanced by  $\omega$ -conotoxin, suggesting that the  $H_3$ -activated  $G_{i/o}$  interacts directly with the N-type voltage-sensitive  $Ca^{2+}$  channel, reducing the channel conductance (Blandizzi C *et al.*, 2001). Direct interaction between  $G_{i/o}$  and  $Ca^{2+}$  channels after  $H_3$  activation is also thought to be the mechanism by which histamine reduces NA release from guinea-pig intestinal sympathetic neurones (Blandizzi C *et al.*, 2000) and rat spinal neurones (Celuch SM, 1995).

Although the  $H_3$  receptor is primarily thought to be a presynaptic receptor, there is evidence which highlights its role in other mechanisms.  $H_3$  receptor activation reduces histamine release from rat peritoneal mast cells *in vivo* (Kohno S *et al.*, 1994).  $H_3$  ligands alter monoamine oxidase activity in human brains *in vitro* (Sakurai E *et al.*, 2001).  $H_3$  receptors have been found on monocytes, dendritic cells and macrophages and are thought to modulate cytokine release (Sirois J *et al.*, 2000; Schneider E *et al.*, 2002). Activation of vascular  $H_3$  receptors has been found to cause vasodilation in the mesenteric vascular bed of the cat (Champion HC *et al.*, 1998), and in rabbit cerebral arteries (Ea Kim L *et al.*, 1992). Both preparations showed the  $H_3$ -mediated vasodilation to be endothelium-dependent.

Recent research has indicated a decongestant effect of  $H_3$  antagonists in an *in vivo* cat model of allergic rhinitis (McLeod RL *et al.*, 1999b). The  $H_3$  antagonist was thought to prevent histamine, via  $H_3$  receptors on sympathetic neurones, from reducing the release of the vasoconstrictor NA. Thus endogenous NA was free to maintain a decongestant effect even in the presence of histamine. This study suggested that histamine was responsible for causing nasal blockage via pathways other than those mediated by  $H_1$  and  $H_2$  receptors. In addition, the study highlighted neurones as targets for the treatment of nasal blockage in allergic rhinitis.

### 1.7 The effect of neurones on nasal patency

As mentioned earlier, there are three major neuronal types involved in nasal inflammation: sensory, parasympathetic and sympathetic neurones. The extent to which these different neuronal types contribute to the control of nasal patency before and during an inflammatory response is discussed below.

### 1.7.1 Sensory neurones

Small diameter C fibres innervate arterial and venous blood vessels, submucosal glands and the epithelium (Baraniuk JN, 1992). Activation of sensory fibres leads to the release of vasoactive neuropeptides, such as substance P, NKA and CGRP. Most studies show that exogenous substance P causes nasal blockage (Devillier P *et al.*, 1988; Braunstein G *et al.*, 1991; Chatelain C *et al.*, 1995; Fajac I *et al.*, 1995; Konno A *et al.*, 1996). CGRP has also been shown to cause nasal blockage (Rangi SP *et al.*, 1990a; Chatelain C *et al.*, 1995), whereas NKA is unable to change nasal patency (Braunstein G *et al.*, 1991). However, acute capsaicin nasal treatment, which stimulates the release of neuropeptide stores from C fibres, causing pain and glandular activation, only occasionally causes nasal blockage (Barnes PJ, 1990; Rajakulasingam K *et al.*, 1992; Sanico AM *et al.*, 1997). Perhaps in studies which fail to show an effect on nasal blockage, capsaicin released neuropeptides in levels too low to cause any significant change in nasal patency.

Phosphoramidon is an inhibitor of neutral endopeptidase, an enzyme responsible for the metabolism of neuropeptides and kinins. Nasal challenge with phosphoramidon had no effect on nasal patency (at a dose which potentiated bradykinin-induced nasal blockage) (Turner PJ, 1999), indicating that neuropeptides are not significantly released in the resting nasal cavity.

In man, neuropeptides appear to play no role in the control of nasal patency in the absence of an inflammatory reaction, and a limited, if any, role in the control of nasal patency after allergen challenge. However, the recent development of selective antagonists (Giardina GA *et al.*, 2003) may lead to greater understanding of the contribution made by neuropeptides to nasal patency.

### 1.7.2 Parasympathetic neurones

Nasal parasympathetic neurones (containing both ACh and VIP) mainly innervate submucosa glands, where they promote secretion. However, parasympathetic fibres also innervate the vasculature and both muscarinic and VIP receptors have been found on nasal blood vessels (Okayama M *et al.*, 1993; Kaliner MA, 1994).

Parasympathetic control of glandular secretion is well documented but its contribution to the control of nasal patency is more debatable. Vidian neurectomy (section of parasympathetic supply to the nasal mucosa) in man has no effect on nasal patency (Konno A *et al.*, 1979). Nasal administration of methacholine has been shown to either produce minor nasal blockage in man (Devillier P *et al.*, 1988; Doyle WJ *et al.*, 1990), or have no effect at all on nasal patency (Zanoletti E *et al.*, 1999). Atropine does not reduce allergen-induced nasal blockage (Georgitis JW, 1998), nor does it have any effect on nasal patency in the unchallenged nose (Baroody FM *et al.*, 1994). However, one study investigating the long-term treatment of PAR using an ipratropium nasal spray reported that the muscarinic antagonist "can contribute to the control of congestion, postnasal drip, and sneezing" (Kaiser HB *et al.*, 1995).

VIP, found in nasal secretions after allergen challenge, is thought to be a potent vasodilator (Baraniuk JN, 1992). *In vitro* studies show that VIP causes nasal vasodilation in cats (Anggard A, 1977) and dogs (Lung MA *et al.*, 1984; Ichimura K *et al.*, 1988b). The single human study with nasal administration of exogenous VIP showed it to cause significant nasal blockage (Chatelain C *et al.*, 1995). In addition, in a study into the role of neuropeptides on headaches in man, a rise in venous blood VIP correlated with a rise in nasal blockage (Edvinsson L, 2001).

Mediators released from parasympathetic neurones may also alter nasal patency indirectly via affecting the release of vasoconstricting mediators from nasal sympathetic neurones (Wang HW *et al.*, 1988).

It seems likely that if the parasympathetic does play a role in the control of nasal patency, it is not via the action of ACh but through VIP-induced vasodilation of nasal blood vessels. However, without safe VIP antagonists, the role of VIP in human nasal blockage will remain elusive.

### 1.7.3 Sympathetic neurones

Nasal sympathetic neurones contain either NA and NPY or NA alone (Baraniuk JN, 1998). Both are potent vasoconstrictors (Wang SH *et al.*, 1993; Baraniuk JN, 1998) and electrical stimulation of sympathetic neurones causes decreased nasal blood

flow and decongestion in the pig (Lacroix JS *et al.*, 1988b), cat (Malm L, 1977; Lacroix JS *et al.*, 1994a), rat (Kawarai M *et al.*, 2001) and dog (Wang M *et al.*, 2003). NPY causes nasal decongestion when applied to human nasal mucosa *in vivo* (Baraniuk JN *et al.*, 1992) and an antagonist at the first NPY receptor ( $Y_1$ ) reduced the vasoconstriction caused by electrical stimulation of sympathetic neurones in pig renal arteries (Lundberg JM *et al.*, 1995).

Much of the evidence in the literature points to NA-induced vasoconstriction being mediated via activation of  $\alpha$ -adrenoceptors (Mygind N, 1982). Both  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors have been shown in human nasal mucosa (van Megen YJ *et al.*, 1991) and  $\alpha$ -adrenoceptor agonists such as NA and phenylephrine contract human nasal arteries *in vitro* (Johannssen V *et al.*, 1997). *In vitro* and *in vivo* studies in rats (Kristiansen AB *et al.*, 1993; Kawarai M *et al.*, 2001) and man (Ichimura K *et al.*, 1988a; Johannssen V *et al.*, 1997) appear to show the NA-induced vasoconstriction is mediated mainly by  $\alpha_1$ -adrenoceptors as it is sensitive to prazosin and phentolamine but not to any great extent to  $\alpha_2$ -adrenoceptor antagonists.

A recent study investigating adrenergic mechanisms in the nasal mucosa of the dog suggested a role for  $\beta$ -adrenoceptors in the throttle veins which drain the sinusoids of their contents (Wang M *et al.*, 2003). Activation of the  $\beta$ -adrenoceptors by NA would lead to dilation of these drainage vessels, thus potentially facilitating the reduction in size of the sinusoids, resulting in further decongestion. However, studies in man show that  $\beta$ -adrenoceptor agonists, when nasally administered, have no effect on nasal patency (Svensson G *et al.*, 1980; Konno A *et al.*, 1987), although a reduction in  $\beta$  adrenoceptors in atopic individuals has been reported (van Megen YJ *et al.*, 1991).

Agents which activate  $\alpha$ -adrenoceptors have been used as powerful decongestants for many years; drugs such as oxymetazoline, xylometazoline and phenylephrine can decrease nasal resistance in the unchallenged nose as well as after allergen challenge (Atkinson TP *et al.*, 1995; Bickford L *et al.*, 1999; Hochban W *et al.*, 1999).

Although the use of sympathomimetics shows the potentially powerful effect of  $\alpha$ -adrenoceptor activation on nasal blockage, it does not, however, indicate the role of

endogenously-released NA on the control of nasal patency. Nevertheless, evidence suggests that the sympathetic nervous system plays an active role in maintaining nasal patency:

- 1) Pharmacological blockade of  $\alpha$ -adrenoceptors/noradrenaline release. Nasal blockage is a significant side-effect of various drugs used for the treatment of hypertension and benign prostatic obstruction, such as  $\alpha_1$ -adrenoceptor antagonists (Moyer JH *et al.*, 1953; Moser M, 1958; Caine M *et al.*, 1981; Kirby RS, 1999), guanethidine (Ferguson RK *et al.*, 1976), methyldopa (Le Gras MD *et al.*, 1990) and reserpine (Murr G, 1976).
- 2) Surgical section of sympathetic neurones. Nasal blockage is a significant side-effect after cervical sympathectomy (Whittet HB *et al.*, 1988; Eccles R, 2000). Increased nasal blood flow is also reported in cervical sympathectomy in rats, cats and dogs (Kawarai M *et al.*, 2001). However, sympathectomy in the guinea-pig caused an acute reduction in nasal symptoms, including nasal blockage (Irifune M *et al.*, 1993).
- 3) Exercise-induced nasal decongestion. Increased sympathetic activity leads to greater release of vasoconstricting mediators from nasal neurones, causing decongestion. Circulating mediators (such as adrenaline) are thought only to contribute marginally to the decongestion (Konno A *et al.*, 1982; Juto JE *et al.*, 1984; Lacroix JS *et al.*, 1997).
- 4) Nasal cycle. Periodic spontaneous changes in asymmetric airflow occur in 21% - 39% of the population (Hanif J *et al.*, 2000). This reciprocating alternation of decongestion/congestion between the two nasal cavities is called the nasal cycle. Changes in nasal patency during the cycle mimic changes in nasal sympathetic activity in the cat. Additionally, in man, the nasal cycle can be altered by electrical stimulation of the hypothalamus (Kennedy B *et al.*, 1986) or abolished by sympathectomy (Baraniuk JN, 1992).



### 1.7.3.1 Modulation of sympathetic synaptic activity by inflammatory mediators

A review of the available literature will show that NA release from sympathetic neurones can be modulated by many substances, for example prostaglandins, kinins, purines, somatostatin, opioids, ACh, histamine and, of course, NA itself (Llona I *et al.*, 1991; Danko G *et al.*, 1994; Boehm S *et al.*, 1997).

Of particular interest for studying the control of nasal patency are the actions of histamine and ACh on nasal sympathetic activity. In a canine model of allergic rhinitis, electrical stimulation of the sympathetic supply to the nasal mucosa led to an increase in NA. This release was reduced by ACh, an effect antagonized by atropine (Jackson RT *et al.*, 1985). Thus, ACh could indirectly promote nasal blockage by inhibiting NA release.

Recently a group at the Schering-Plough Research Institute, Kenilworth, New Jersey, published a paper describing a novel H<sub>3</sub> agonist, similar in potency to R- $\alpha$ -methylhistamine. One of the characteristics of this new drug, Sch 50971, was that it "inhibits the effects of sympathetic nerve stimulation on nasal resistance in cats" (Hey JA *et al.*, 1998), indicating that activation of H<sub>3</sub> receptors caused nasal blockage. This identified an inhibitory H<sub>3</sub> receptor on nasal sympathetic neurones, a discovery not completely surprising as presynaptic H<sub>3</sub> receptors have been found on many autonomic neurones previously.

A year later, the group showed that H<sub>3</sub> antagonists could reduce the nasal blockage caused by compound 48/80 when administered nasally to cats (McLeod RL *et al.*, 1999b). However, this effect on the nasal blockage only occurred when in combination with an H<sub>1</sub> antagonist. The H<sub>1</sub> and H<sub>3</sub> antagonist combination was later shown to be acting peripherally as antagonists which failed to cross the blood brain barrier were still effective in reducing nasal blockage (McLeod RL *et al.*, 2003).

*In vitro* electric field stimulation-induced vasoconstriction in porcine nasal mucosa is also inhibited by both R- $\alpha$ -methylhistamine and compound 48/80, an effect reduced by the H<sub>3</sub> antagonists thioperamide and clobenpropit. R- $\alpha$ -methylhistamine did not, however, inhibit exogenously-applied NA-induced vasoconstriction, indicating the H<sub>3</sub> receptor is presynaptic (Varty L *et al.*, 2002).

The recent *in vivo* and *in vitro* data in the cat and pig suggests that histamine is able to cause vasodilation, and thus nasal blockage, by a direct H<sub>1</sub>-mediated mechanism and an indirect H<sub>3</sub>-mediated mechanism. Combined H<sub>1</sub>/H<sub>3</sub> vasodilation has also been reported in guinea-pig ileum *in vitro*; again the H<sub>1</sub>-mediated vasodilation was a direct effect on the vascular endothelium and activation of the H<sub>3</sub> receptor inhibited NA release, thus augmenting vasodilation (Beyak M *et al.*, 1995).

Until early 2004, no studies focusing on the role of H<sub>3</sub> in human nasal blockage had been published. In January 2004, the Schering-Plough group published a paper investigating human nasal H<sub>3</sub> receptors *in vitro* (Varty LM *et al.*, 2004). Firstly, the study showed high levels of H<sub>3</sub> receptor mRNA in human nasal mucosa. Secondly, the paper demonstrated that *in vitro* electric field stimulation-induced vasoconstriction in human nasal mucosa is inhibited by R- $\alpha$ -methylhistamine, an effect reduced by the H<sub>3</sub> antagonist clobenpropit. This paper showed that H<sub>3</sub> receptors in human nasal mucosa may participate in the control of nasal blockage in a similar way to that which had been demonstrated in the pig *in vitro* study and the cat *in vivo* studies.

In addition to the evidence supporting a role of H<sub>3</sub> receptors in regulating sympathetic synaptic activity, excitatory H<sub>1</sub> receptors have been shown on human nasal sympathetic neurones, whilst inhibitory H<sub>2</sub> receptors have been demonstrated on dog and guinea-pig nasal sympathetic neurones (Kubo N *et al.*, 1989b). Such evidence suggests a complex, possibly dose-dependant, role of histamine in controlling NA release.

Clearly, indirect H<sub>3</sub>-mediated vasodilation can only occur if NA is basally released. Without NA actively controlling the tone of the blood vessels, any reduction in NA release would have no effect. Quite simply, it is impossible to remove the effect of a vasoconstrictor that is not released in the first place.

## 1.8 Hypothesis

The sympathetic nervous system plays a key role in the maintenance of human nasal vascular tone in both the unstimulated and stimulated nasal mucosa. In addition, histamine can cause nasal blockage in man, directly (H<sub>1</sub>-mediated) and

indirectly via the H<sub>3</sub>-mediated modulation of sympathetic outflow. Manipulation of sympathetic activity represents a suitable target for the treatment of nasal blockage. The hypothetical mechanisms by which histamine can cause nasal blockage is summarized in Figure 1.2.

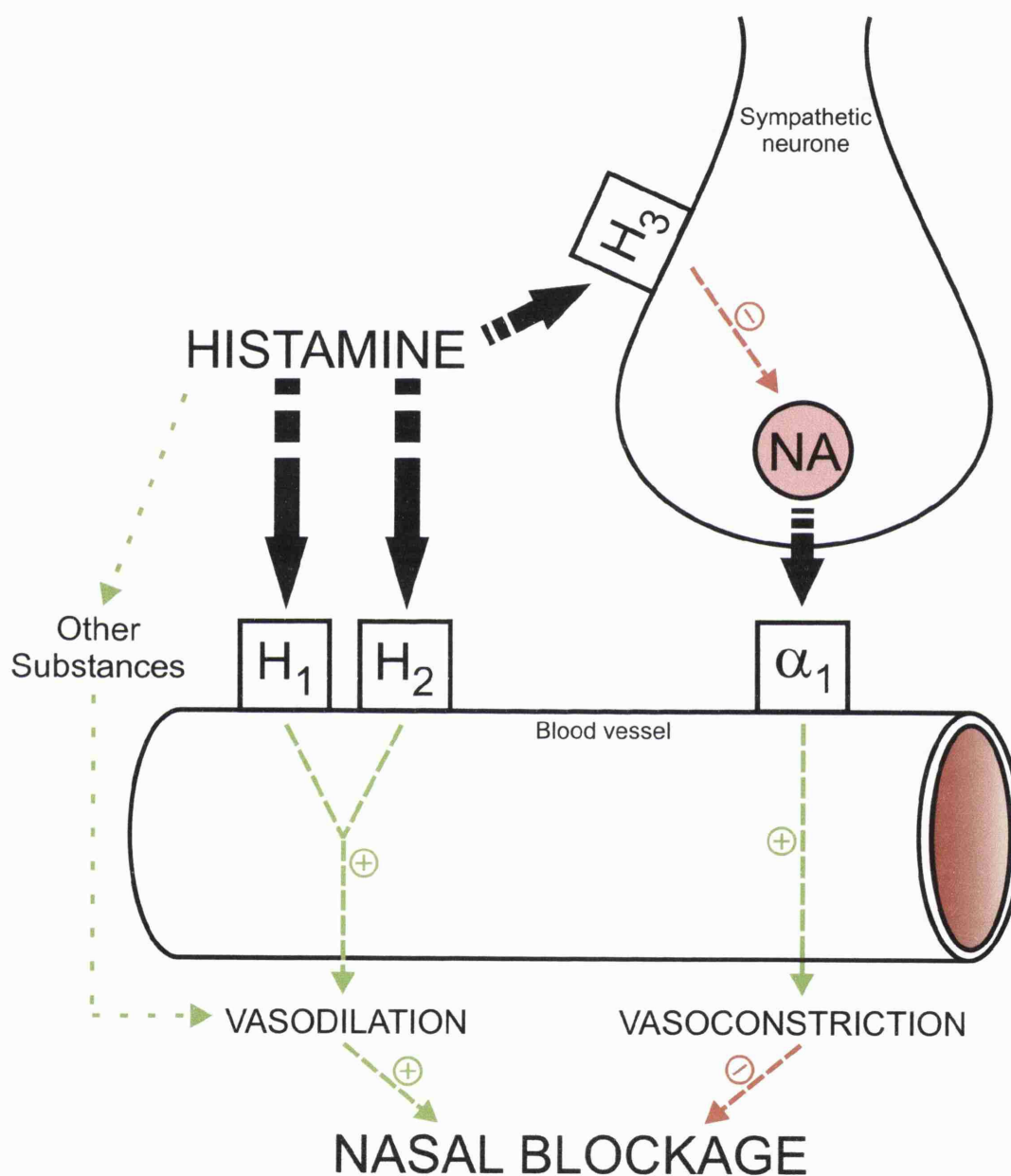


Figure 1.2: Hypothetical schematic showing the role of H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors in the control of human nasal blockage. Green arrows denote activation, red arrows denote inhibition.

## **1.9 Aims of the project**

- 1) Investigate the effect of histamine receptor agonists on nasal patency.
- 2) Establish the degree to which histamine receptor antagonists reduce nasal blockage.
- 3) Investigate the role played by the sympathetic nervous system in the control of nasal patency.

# CHAPTER 2

## MATERIALS AND METHODS

### 2.1 Materials

The materials used in the following studies are shown below:

<u>MATERIAL</u>	<u>SOURCE</u>
<b>Pharmacological agents</b>	
Bradykinin	Merck Biosciences, Nottingham, UK.
Cetirizine (tablets)	Lagap, Hampshire, UK.
Chlorpheniramine	Sigma, Poole, UK.
Chlorpheniramine (tablets)	Allen & Hanburys, Middlesex, UK.
Clonidine	Sigma, Poole, UK.
Corynanthine	Sigma, Poole, UK.
Dimaprit	Tocris, Avonmouth, UK.
Famotidine (tablets)	Johnson & Johnson, Buckinghamshire, UK.
Grass pollen mixed allergen	Hal Allergen Laboratory, Duesseldorf, Germany.
Histamine	Sigma, Poole, UK.
Lidocaine	Sigma, Poole, UK.
Noradrenaline	Sigma, Poole, UK.
Ranitidine (tablets)	GlaxoSmithKline, Stevenage, UK.
R- $\alpha$ -methyhistamine	Tocris, Avonmouth, UK.
S- $\alpha$ -methylhistamine	Tocris, Avonmouth, UK.
Thioperamide	Tocris, Avonmouth, UK.
Xylometazoline	Novartis Consumer Health, Horsham, UK.
<b>Assay reagents</b>	
Acetonitrile	Sigma, Poole, UK.
Citric acid	Sigma, Poole, UK.
Ethylenediaminetetraacetic acid	Sigma, Poole, UK.
HPLC-grade water	Fischer, Leicester, UK.
Methanol	Merck Biosciences, Nottingham, UK.

NaH <sub>2</sub> PO <sub>4</sub>	Fischer, Leicester, UK.
Octane sulphonic acid	Sigma, Poole, UK.
Triethylamine	Sigma, Poole, UK.

### Miscellaneous

Catecholamine extraction Kit	ESA Analytical, Buckinghamshire, UK.
Kapiseal Earseals	Kapitex Healthcare, Yorkshire, UK.

## 2.2 Methods for functional studies

### 2.2.1 Dilution of nasal challenge agents

All compounds were dissolved in sterile saline (NaCl 154mM) to make stock solutions, which were further diluted and divided into appropriate aliquots (typically 1.2ml to 1.5ml) and stored in 7ml bijoux at -20°C. Aliquot concentrations for each compound were as follows: histamine, 0.4mg/ml to 10mg/ml; dimaprit, 0.5mg/ml to 2mg/ml; R- $\alpha$ -methylhistamine, 1mg/ml to 6mg/ml; S- $\alpha$ -methylhistamine, 6mg/ml; chlorpheniramine, 10mg/ml; thioperamide, 7mg/ml; bradykinin, 1mg/ml to 2mg/ml; lidocaine, 40mg/ml; clonidine, 1mg/ml; corynanthine, 20mg/ml; grass pollen mixed antigen, 5000U/ml.

All dilutions were performed in a class II microbiological safety cabinet. To aid the dissolving of some of the compounds, a vortex and a sonicator were used. For light-sensitive compounds, such as chlorpheniramine and corynanthine, tin foil-wrapped bijoux were used to protect the solutions from degradation and the dilutions were performed in minimal light. All aliquots were sufficiently large enough to allow for nasal challenge of only one subject. Aliquots were defrosted approximately 30 minutes before use, and were gently raised to 37°C in a water bath.

Grass pollen mixed antigen was composed of *Agrostis stolonifera*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Poa pratensis*, *Secale cereale*, *Phleum pratense* and *Hordeum vulgare* in equal concentrations.

### 2.2.2 Subjects

For all studies, except those investigating allergen challenges in atopic individuals (Chapter 5), normal, healthy, non-atopic volunteers within the age-range 19 to 54 years were used. No subject had a clinical history of allergic disease or any nasal pathology. For the allergen challenge studies, only subjects (aged 19 to 54) with a history of seasonal allergic rhinitis and a positive skin prick test to grass pollen were used. Subjects with a positive skin prick test to house dust mite, *Dermatophagoids pteronyssinus*, were excluded. Subjects with a history of asthma were also excluded.

All subjects, both atopic and healthy volunteers, took no medication at the time of, or in the four weeks preceding the experiments. All subjects gave informed consent and the studies were all approved by the relevant local Ethics Committee.

The number of subjects used in each study was based on the estimated expected difference in nasal response between treatment groups.

### 2.2.3 Acoustic rhinometry

Acoustic rhinometry is a well-used research technique for objectively measuring nasal blockage (Austin CE *et al.*, 1994c; Fisher EW *et al.*, 1994). The acoustic rhinometer (supplied by GM instruments, Kilwinning, UK) produces a click which travels up a hollow tube, through a 6cm sterile plastic nose piece, and into the subject's nasal cavity. The sound reflects off the internal structures of the nasal cavity and back down the tube to the internal microphone of the acoustic rhinometer. The signal is amplified and sent to a computer. The Nasal Area Distance Acquisition Program calculates the internal cross-sectional area along the length of the subject's nasal airways (Figure 2.1).

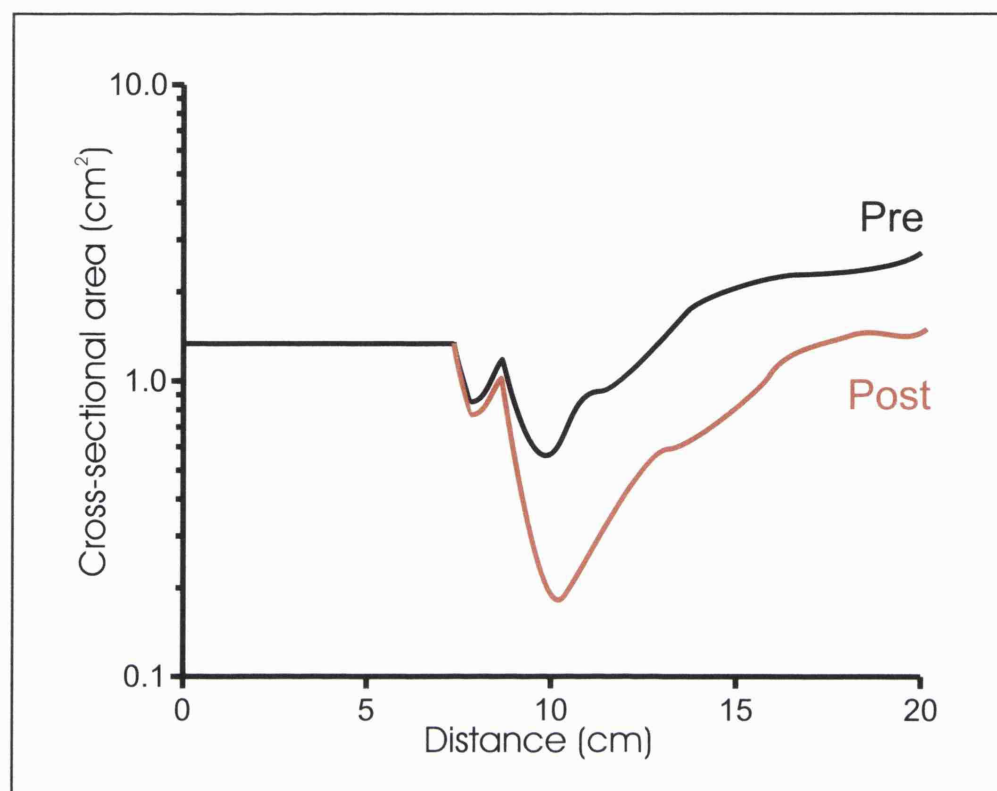


Figure 2.1: Typical output from the Nasal Area Distance Acquisition Program following measurement of a subject's nasal cavity by acoustic rhinometry. x-axis = distance (cm) along the length of the nose piece and the nasal cavity; y-axis = cross-sectional area ( $\text{cm}^2$ ) of the cavity at a given distance (log scale). Figure shows the change in cross-sectional area along the cavity following nasal challenge with an inflammatory substance. Pre = trace recorded before nasal challenge, Post = trace recorded after nasal challenge.

This technique is non-invasive and requires virtually no subject training; although the reproducibility of measurements was improved if the subject maintained the correct posture during the 2 seconds of measurement: sitting on a stable stool with a straight back, facing front with a closed mouth. This posture was easier to maintain if the subject first took a deep breath.

The minimum cross-sectional area ( $A_{\text{min}}$ ) between 1.2 and 7cm from the nasal orifice, corresponding to the location of the inferior and middle turbinates, was recorded as the objective measurement of nasal blockage. Both nostrils were measured separately three times at each time point. For each time point an overall mean nasal  $A_{\text{min}}$  was then calculated.



As mentioned in Chapter 1.5.2.3, acoustic rhinometry is a quick and reliable method of objectively measuring nasal blockage. Figure 2.2 shows the baseline Amin. data (prior to nasal challenge) measured from six individual subjects (A to F) who participated in many of the studies. Over the course of three years of experimentation, the mean coefficient of variance for these subjects was 10.75% ( $\pm 1.25\%$ ).

## 2.2.4 Symptom scores

As an objective measurement of sneezing, the number of sneezes was recorded by the investigator. For other nasal symptoms, such as nasal congestion, pruritus and rhinorrhea, the subjects themselves graded these symptoms using either visual analog scores (VAS) or an integer scoring system. Using VAS (Figure 2.3), a horizontal line representing the possible spectrum of symptom severity, with the left side representing no symptom and the right, the worst imaginable, was marked by the subject, based on their assessment of their symptom severity. The distance (mm) of the mark from the left of the horizontal line was then recorded as the subject's symptom score.

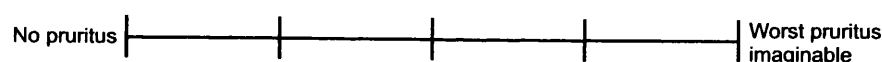


Figure 2.3: Example of a visual analog score (VAS) for nasal pruritus.

The integer scoring system uses a scoring system of 0 to 5, with 0 representing no symptom and 5, the worst imaginable. Subjects graded each symptom either 0, 1, 2, 3, 4 or 5 depending on their assessment of their symptom severity.

## 2.2.5 Nasal challenge

The pharmacological agents were administered to the nasal cavity via a hand-held nasal pump 100 $\mu$ l spray (Perfect-Valois, UK Ltd). The spray was placed inside the subject's nostril and activated once, thus delivering 100 $\mu$ l of solution. Xylometazoline nasal challenge was performed using a pump spray supplied by the manufacturer, also delivering 100 $\mu$ l of solution. In all studies, unless stated otherwise (e.g. Chapter 3.2.2), both nostrils were sprayed for each nasal challenge. The dose administered was controlled by the concentration of the solution. The

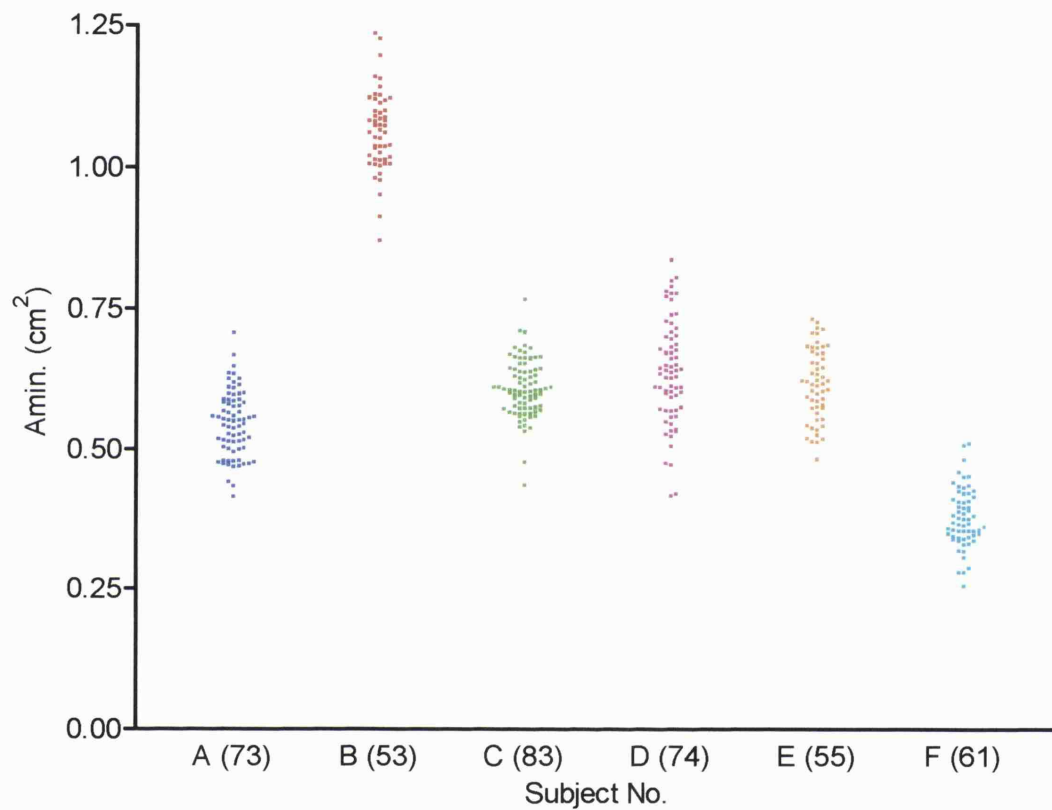


Figure 2.2: The spread of nasal patency in the untreated nose amongst six individuals (Subjects A to F), as measured by minimum cross-sectional area [Amin.]. Each data point represents the mean nasal Amin. calculated from three measurements in each nostril. The number of experiments for each individual is in parentheses. The mean coefficient of variance is 10.75% ( $\pm 1.25\%$ ).

doses stated in the experimental protocols were the amounts administered into each nostril. All solutions were raised to 37°C before nasal challenge. Studies were performed in a laboratory at controlled temperature and humidity.

### 2.2.6 General protocol for nasal challenge experiments

All experiments followed the same double-blind, randomized, cross-over study design. For each study, subjects received all treatments in a random order with only one treatment per day allowed. Thus each subject acted as his/her own control. Acoustic rhinometry was used as an objective measurement of nasal patency. In all studies, unless stated otherwise (e.g. Chapter 3.2.2), assessment of the nasal patency was calculated according to the following method: at each time-point, three Amin. measurements for each nostril were taken and the six values were averaged to give the mean Amin. for that time-point. All experiments used the same basic protocol: baseline Amin. recording, followed by nasal challenge followed by Amin. recordings 5, 10 and 15 minutes later (Figure 2.4).

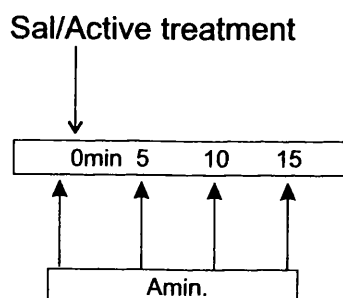


Figure 2.4: General protocol for nasal challenge experiments. Sal = saline.

In the studies including oral treatments, both active treatment (e.g. H<sub>1</sub> antagonist) and placebo were given 2 hours prior to nasal challenge. Every attempt was made to administer oral treatments when the subject had an empty stomach.

In the studies investigating pollen challenge in atopics, experiments were performed out of the pollen season (e.g. late autumn), so as to control allergen exposure.

### 2.2.7 Data analysis

Although the data analysis for each study is described in the relevant results chapter, there are some common aspects to nearly all studies. In nearly all studies, subject Amin. was measured, followed by nasal challenge followed by Amin. recordings 5, 10 and 15 minutes later, as shown in Figure 2.4. An example of the changes in an individual subject's Amin. following different treatments plotted against time is shown in Figure 2.5. The units for Amin. are  $\text{cm}^2$ . In this example the subject has undergone three separate experiments, each on a different day. The three treatments are saline nasal challenge, histamine nasal challenge and histamine nasal challenge following pretreatment with an oral dose of cetirizine, the  $H_1$  antagonist. As shown in Figures 2.2 and 2.5, there is both intra- and inter-subject variation in baseline Amin. recordings. By normalising the Amin. data recorded after nasal challenge ( $t = 5, 10$  and  $15$  minutes) to the pre-challenge value, the small intra-subject baseline variation is eliminated, as shown in Figure 2.6. The units for normalised Amin. are arbitrary units. By calculating the area under the curve (AUC) for each treatment, the data for the four time-points ( $t = 0, 5, 10$  and  $15$  minutes) were combined into one single value which represents the overall nasal response for that treatment for that subject. In the example shown in Figure 2.7, the AUC for saline, histamine and histamine following pretreatment with cetirizine are calculated as 15.21units.min, 7.96units.min and 13.97units.min, respectively. By analyzing the AUC of normalised Amin. data, it is easy to gauge the effect of a given treatment on nasal patency: no change in nasal patency following nasal challenge would produce an area under the normalised Amin. response versus time curve of 15units.min (normalised Amin. of 1 for each time-point, for the duration of 15 minutes). A value less than 15units.min would indicate a decrease in nasal patency, and thus nasal blockage; a value greater than 15units.min would indicate an increase in nasal patency.

The consistency and reproducibility of this method is shown in Figure 2.8 and 2.9. Figure 2.8 shows the effect of saline and histamine, 400 $\mu\text{g}$ , on the area under the Amin. response versus time curve for six subjects (A to F), as measured by acoustic rhinometry in 6 separate experiments. Although the intra-subject variation in nasal response to saline and histamine is relatively low (mean coefficient of variance = 14.9% (+/- 2.47%)), there is a large inter-subject variation (coefficient of

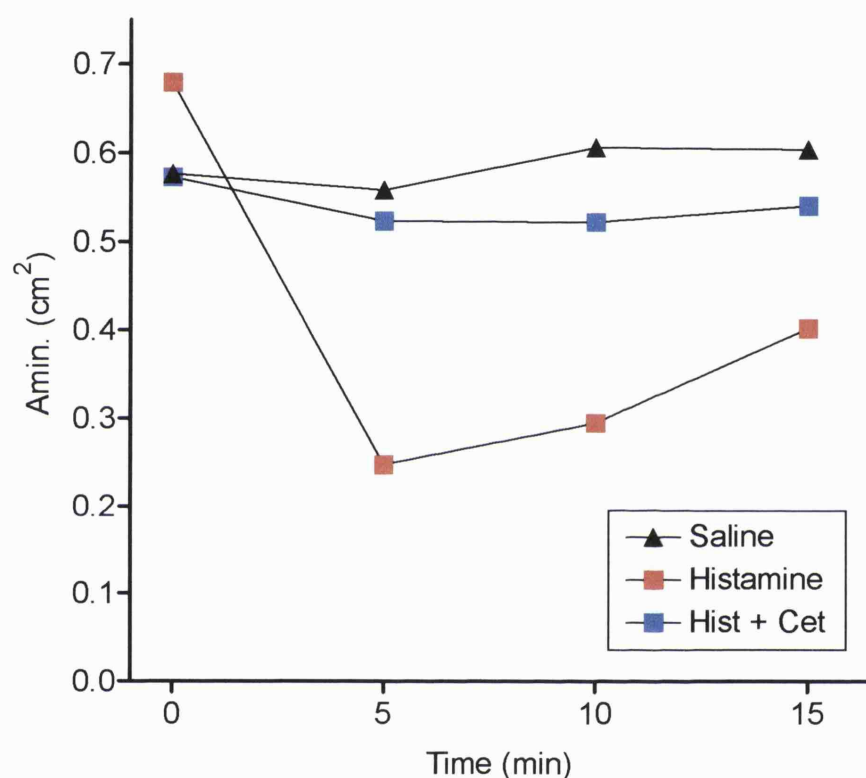


Figure 2.5: The effect of nasal administration of saline [▲] or histamine (alone [■]) or pretreated with cetirizine [■]) on the Amin. recorded from the same individual over a period of 15 minutes following nasal challenge. Amin. was measured immediately before, and 5, 10, and 15 minutes after nasal challenge. Cetirizine [Cet], 20mg, was given orally 2 hours prior to challenge with histamine. Both saline and histamine [Hist], 400µg, were administered as aerosols into each nostril.

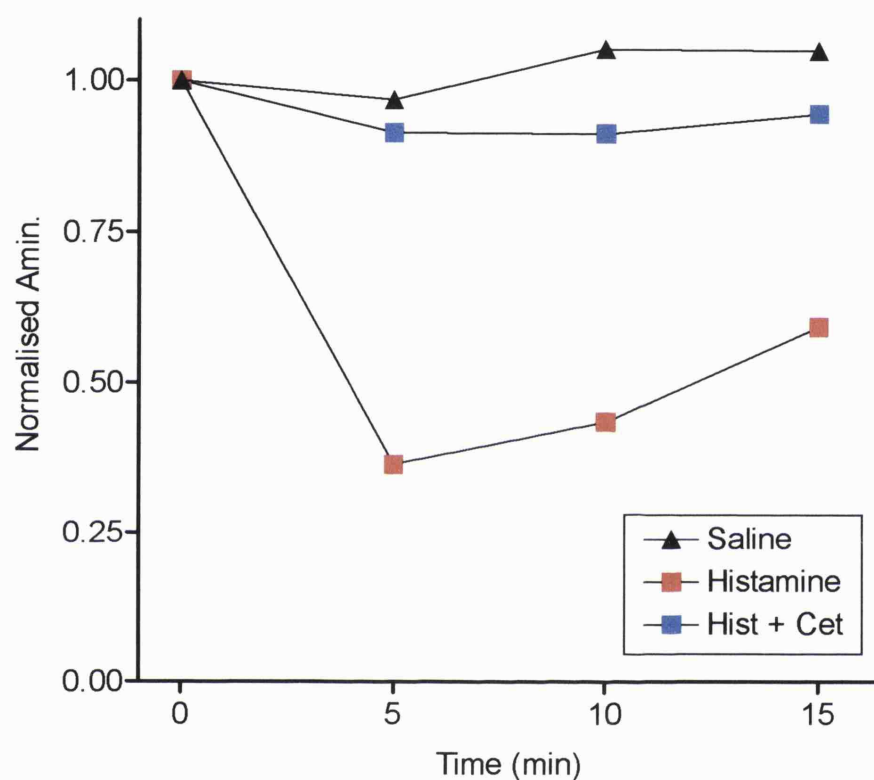


Figure 2.6: The effect of nasal administration of saline [▲] or histamine (alone [■]) or pretreated with cetirizine [■]) on the Amin. recorded from the same individual over a period of 15 minutes following nasal challenge. Amin. was measured immediately before, and 5, 10, and 15 minutes after nasal challenge. Amin. values were normalised to the pre-challenge value (mean of  $0.609\text{cm}^2$ , s.e.mean of  $0.030\text{cm}^2$ ). Cetirizine [Cet], 20mg, was given orally 2 hours prior to challenge with histamine. Both saline and histamine [Hist], 400 $\mu\text{g}$ , were administered as aerosols into each nostril.

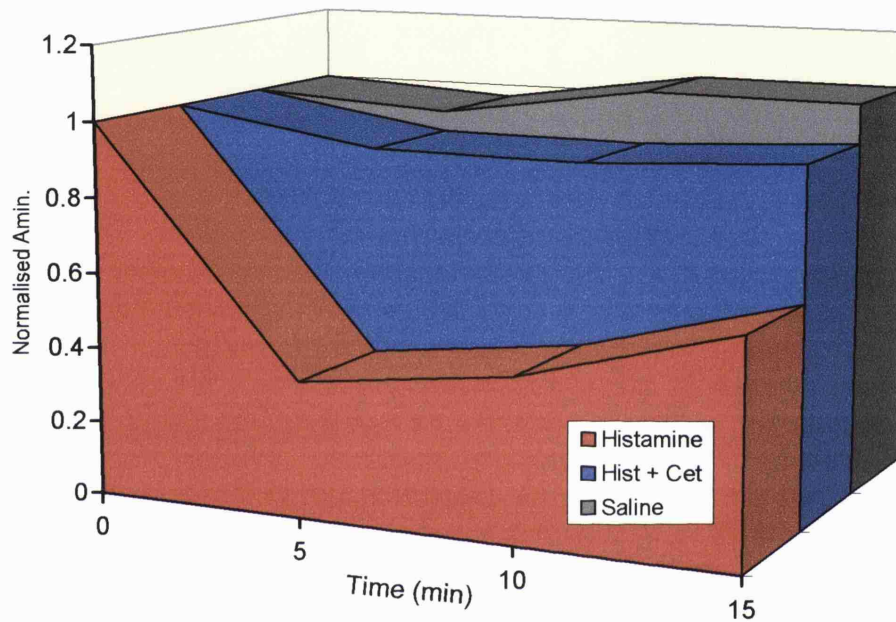


Figure 2.7: The effect of nasal administration of saline [■] or histamine (alone [■]) or pretreated with cetirizine [■]) on the Amin. recorded from the same individual over a period of 15 minutes following nasal challenge. Amin. was measured immediately before, and 5, 10, and 15 minutes after nasal challenge. Amin. values were normalised to the pre-challenge value (mean of  $0.609\text{cm}^2$ , s.e.mean of  $0.030\text{cm}^2$ ). Cetirizine [Cet], 20mg, was given orally 2 hours prior to challenge with histamine. Both saline and histamine [Hist], 400 $\mu\text{g}$ , were administered as aerosols into each nostril.

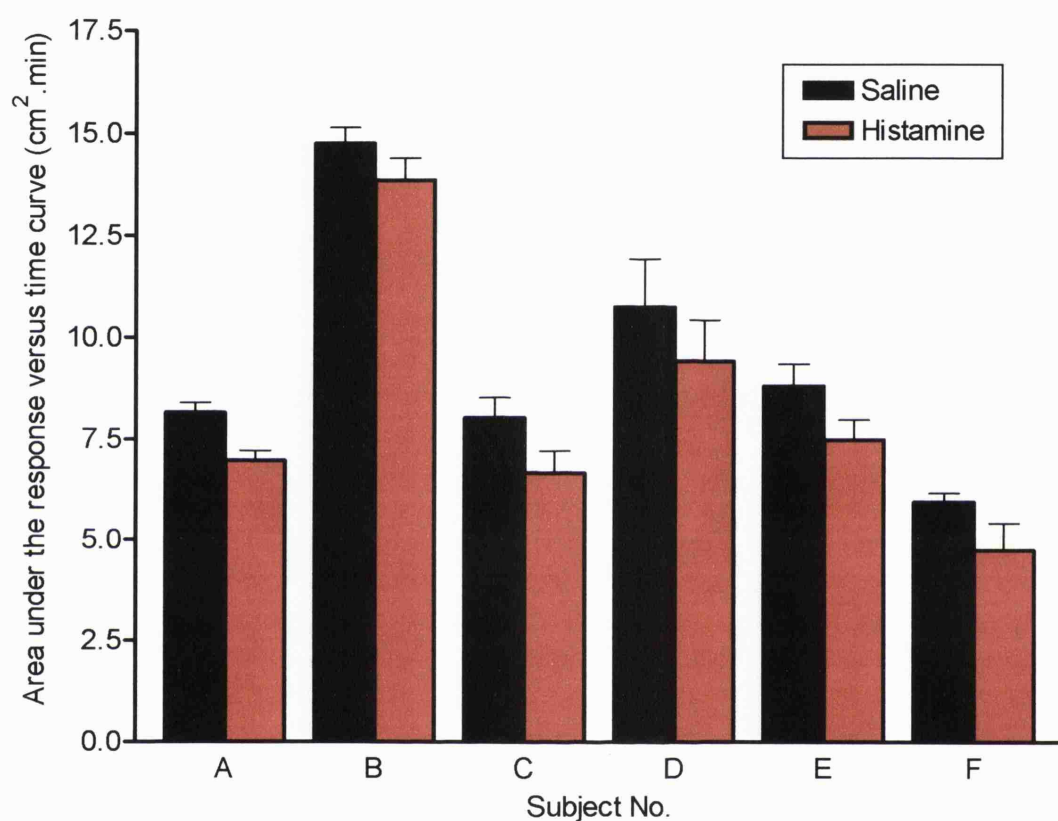


Figure 2.8: The spread between six individuals (Subjects A to F) of the effect of histamine on the area under the Amin. versus time curve measured over a period of 15 minutes following the administration of histamine, 400 $\mu$ g, as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Data are means from 6 experiments. Vertical bars represent the s.e.mean.



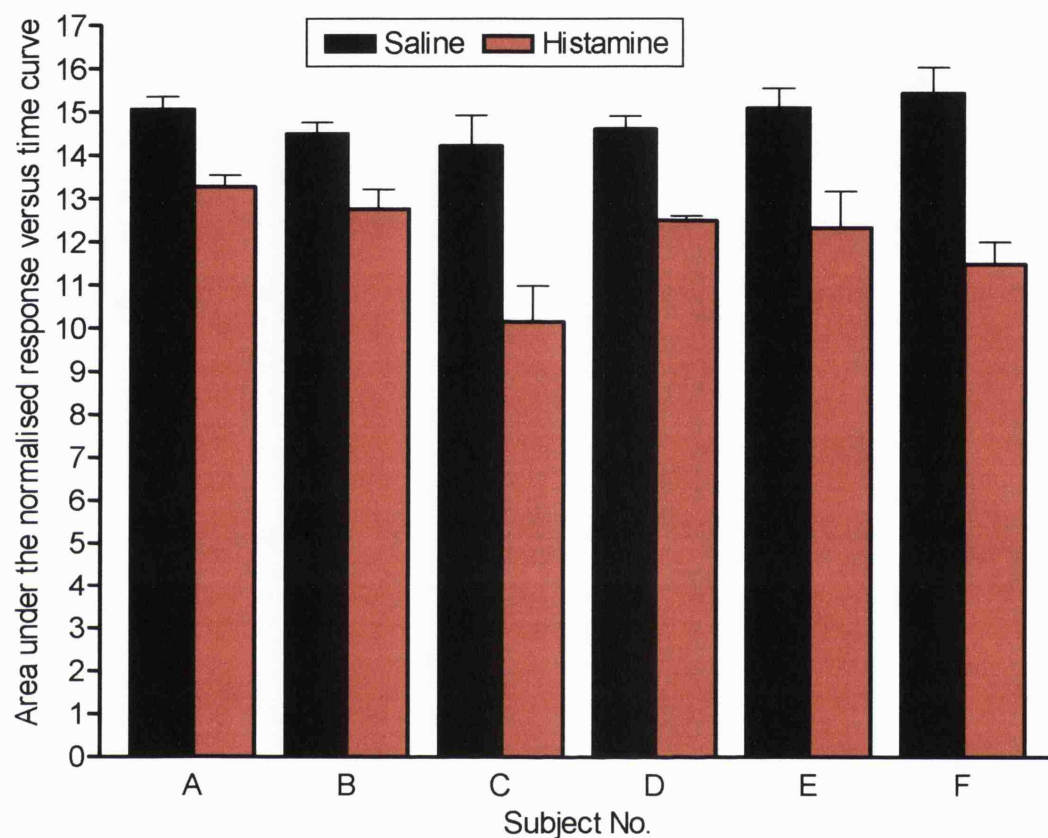


Figure 2.9: The spread between six individuals (Subjects A to F) of the effect of histamine on the area under the normalised Amin. versus time curve measured over a period of 15 minutes following the administration of histamine, 400 $\mu$ g, as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.606cm<sup>2</sup>, s.e.mean of 0.034cm<sup>2</sup>). Data are means from 6 experiments. Vertical bars represent the s.e.mean.

variance = 34.3%), due to the unique nasal topography of each subject, as shown in Figure 2.2. If the data for each experiment is normalised to the baseline  $A_{min}$ , as discussed earlier, both the intra-subject variations (mean coefficient of variance = 8.54% (+/- 1.63%)) and inter-subject variations (coefficient of variance = 15.3%) are reduced. Figure 2.9 shows the effect of saline and histamine, 400 $\mu$ g, on the area under the normalised  $A_{min}$  response versus time curve for the same six subjects (A to F), as measured by acoustic rhinometry in the same 6 experiments. It is interesting to note that there is less inter-subject variation with saline challenge compared to histamine challenge (coefficient of variance = 7.56% and 14.2%, respectively). The AUC data for saline challenge represents no change in nasal patency (the nasal cavity is unaffected by isotonic saline), whereas the AUC data for histamine challenge represents an active physiological change in the nasal mucosa. It is not surprising that some subjects react more strongly to histamine, 400 $\mu$ g, than others, thus the inter-subject variation is likely to be greater for active treatments than for the saline control.

### 2.2.8 Statistical analysis

The data from all the nasal challenge studies were analysed using non-parametric statistical tests to determine the probability of rejecting the null hypothesis. In all studies, the null hypothesis stated that there was no difference in nasal responses between control and active treatments. Non-parametric analyses were used because, firstly, the data did not always follow normal Gaussian distribution and, secondly, as mentioned in Chapter 2.2.7, the standard deviation of active treatment groups was often greater than that of control groups.

For each study, a power calculation was performed in order to determine the required number of experimental subjects. This calculation was based on the estimated expected difference in nasal response between treatment groups, the estimated standard deviation of nasal responses, an  $\alpha$  value of 0.05 and a  $\beta$  value of 0.2.

In all experimental studies, each subject received all the treatments, thus the statistical tests used were *repeated measures* tests. When two groups were compared, for example histamine challenge compared with histamine challenge

following pretreatment with an H<sub>2</sub> antagonist, the Wilcoxon sign-rank test was used. When more than two groups were compared, for example a range of drug concentrations, the Friedman's test was used. The Spearman's rank test was used to compare two groups of variables, so as to determine any correlation between the two groups. For the meta-analysis in Chapter 4, as not every treatment group contained the same subjects, when more than two groups were compared, the *non-repeated measures* version of the Friedman's test, the Kruskal-Wallis test, was used with post-hoc Dunn's multiple comparisons test comparing groups within the analysis. A probability, *p*, of less than 0.05 was taken as significant.

## 2.3 Methods for catecholamine assays

### 2.3.1 Nasal lavage

During a nasal lavage, the nasal cavity is washed out with isotonic saline, and substances from the cavity are collected with the recovered saline. The technique requires minimal subject training and is well tolerated. The method used was developed from one described by Turner (1999). The method involves the gentle syringing of each nasal cavity with warmed saline (154mM NaCl), either 2.5ml or 5ml, using a syringe with a nasal olive (made from earseals supplied by Kapitex Healthcare Ltd, Yorkshire, UK) attached to its tip. The nasal olive prevents the instilled liquid leaking out of the cavity during the process. The subject sits on a stable stool, with his/her head tipped forward. The subject holds the syringe vertically with the tip facing upwards. The subject gently places the tip into one nostril so that the nasal olive forms a seal in the nostril aperture. The saline is then instilled into the nasal cavity and left for 10 seconds. The subject then quickly removes the syringe from the nostril and the investigator collects the lavage fluid as it drains from the nasal cavity. The lavage fluid is filtered using a 0.9mm sieve and collected in a 30ml container. A lavage is performed in each cavity separately, then collected in the same 30ml container. The volume of recovery from each lavage is typically ~80%. In some cases, the nasal cavities of a subject were lavaged three times, with only the last lavage from each cavity being collected for analysis. This method is employed to prevent the build-up of substance levels prior to the first lavage biasing the data.

### 2.3.2 Sample treatment

The unique process by which the lavage samples were prepared for each study is described in detail in the relevant chapter. The different techniques used in these studies are summarised below:

**Vortex:** solutions were vigorously mixed using a Whirlimixer, manufactured by Fisons, Leicester, UK.

**Centrifugation:** solutions were centrifuged (separating insoluble material from the solution), using a Mistral 3000i centrifuge, manufactured by MSE Scientific Instruments, Crawley, UK.

**Temperature control:** the lavages were collected in chilled 30ml containers, the centrifuge was pre-cooled to 4°C and the processed samples were kept in the refrigerator between data acquisitions.

**pH control:** perchloric acid was used to precipitate out any protein in the sample solution and, in addition, to stabilize noradrenaline (NA) levels.

**Preventing exposure to light:** the samples were stored in opaque amber micro-capped centrifuge tubes or kept in a dark refrigerator between data acquisitions.

**Noradrenaline spike:** known amounts of NA were added to lavage samples in order to act as a positive control.

Using a catecholamine extraction kit (ESA Analytical, Buckinghamshire, UK), lavage samples were filtered through alumina, leaving behind all catecholamines. The catecholamines were then eluted using a non-polar buffer. 1.5ml of sample was added to the extraction tube which contained alumina. The tube was then inverted to mix for 10 minutes. The top and bottom caps were removed and the filtrate was aspirated to waste by vacuum. The extraction tube was filled with saline and the filtrate was again aspirated to waste by vacuum. 200µl of eluting solution was added to the extraction tube. The caps were replaced and the tube was then inverted to mix for 10 minutes. The caps were removed and the solution was forced

from the tube into a pre-cooled opaque amber micro-capped centrifuge tube using a pipette bulb. The tube was then stored at -20°C.

### **2.3.3 High pressure liquid chromatography coupled with electrochemical detection**

Nasal lavages, for the purposes of analysis, can be considered to be similar to physiological solutions in that they are complex mixtures of many substances, some very similar in structure. However, using high pressure liquid chromatography (HPLC), it is possible to separate even closely related substances, so that the accurate detection of specific substances can be made (Aguilar MI, 2004). HPLC has previously been used to separate closely related substances in nasal lavages from human subjects (Ramis I *et al.*, 1988).

The technique used for the separation of NA from the contents of the nasal lavage was reversed-phase ion-pair chromatography (Knox JH *et al.*, 1976) on an octadecylsilane column. Ion-pairs (lavage molecules bonded to the detergent (octane sulphonic acid), passing down the column, form hydrophobic bonds with the silica-based surface. The retention time of a given substance depends on the strength of these hydrophobic interactions. Thus a lavage molecule with a high capacity for hydrophobic interactions will be held in the column longer and will come off the column later than a less hydrophobic molecule. Therefore, the molecules in the lavage were separated according to their structural hydrophobicity. The retention time for a given substance also depends on the pH, the temperature and the concentration and nature of the detergent in the aqueous organic eluent, and these factors were controlled to give a NA peak at around 6 minutes for the in-house HPLC and around 4 minutes for the Pfizer HPLC.

After the separation in the column, the solutes passed into the electrochemical cell where they were then oxidized. The electrochemical cell contains two carbon electrodes, one positively charged, one negatively, and it is at the former where the transfer of an electron from the molecule in solution was recorded as a current by the detector. Given that the solutes were separated by the HPLC, the current recorded was only dependent on the concentration of that solute. Thus, the greater the concentration of the solute, the higher the current. The current was expressed

on a computer chromatogram. The output was in the form of a current versus time graph and so the concentration of the molecule in question is proportional to the peak height and also proportional to the area under that peak. This can be converted into  $\text{mol/dm}^3$  by comparing the peak with a peak derived from a known standard.

### 2.3.3.1 In-house high pressure liquid chromatography coupled with electrochemical detection: equipment and buffer

The in-house HPLC-ECD consisted of the following equipment, set up as shown in Figure 2.10:

Aquapore Guard column; ESA 5014 electrochemical cell (-ve: -280mV; +ve: 180mV); ESA 5100A Coulochem detector; ESA Pulse dampener; Guard cell ESA 5020 (+350mV); Hypersil ODS-5 (octadecylsilane) column; Rheodyne 7125 injection port with 50 $\mu\text{l}$  loop; Shimadzu LC 6A isocratic pump ESA 582 (flow rate 1.6 - 1.8 ml/min); Turbochrom Navigator (computer software).

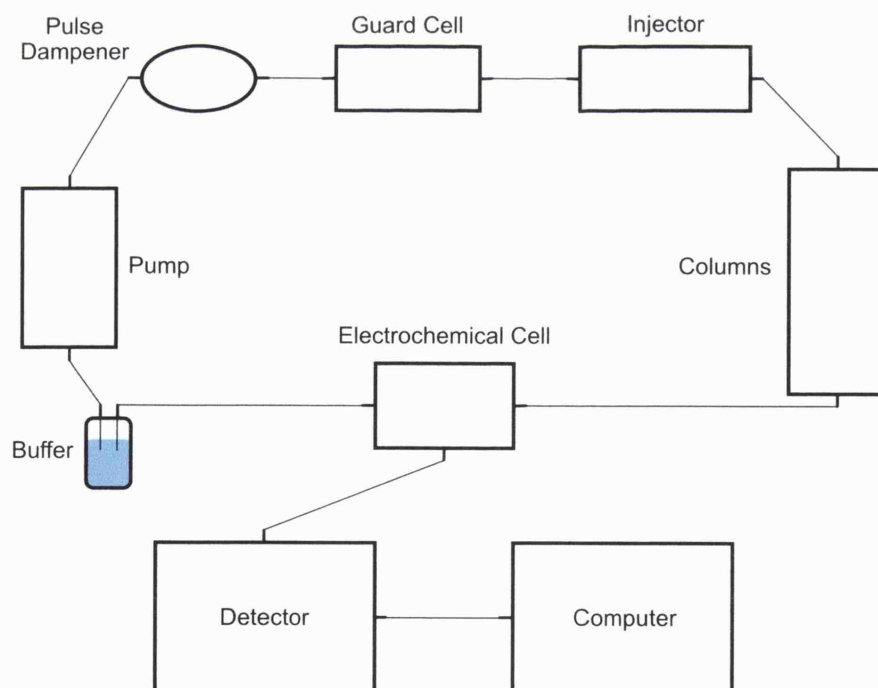


Figure 2.10: In-house HPLC-ECD set up.

The buffer consisted of the following reagents: Octane sulphonic acid, 2mM;  $\text{NaH}_2\text{PO}_4$ , 0.1M; methanol, 12%; ethylenediaminetetraacetic acid, 0.67mM; and HPLC-grade water.

### **2.3.3.2 Pfizer high pressure liquid chromatography coupled with electrochemical detection**

The buffer consisted of the following reagents: octane sulphonic acid, 1.7mM;  $\text{NaH}_2\text{PO}_4$ , 0.09M; acetonitrile, 10%; ethylenediaminetetraacetic acid, 0.05mM; citric acid, 50mM; and HPLC-grade water. Adjusted to pH 3.0 with phosphoric acid

Prior to analysis of the lavages, analyte retention time and concentration/peak height ratios were calibrated using analyte standards. The following substances were measured: 3,4-dihydroxyphenylglycol (DHPG), 3-methoxy, 4-hydroxyphenylglycol (MHPG), noradrenaline, adrenaline, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptophan (5-HTP), dopamine, 5-hydroxyindoleacetic acid (5-HIAA), N-acetyl-5-hydroxytryptamine (N-acetyl-5-HT), homovanillic acid (HVA) and serotonin.

# CHAPTER 3

## THE ROLE OF THE HISTAMINE H<sub>1</sub> RECEPTOR IN HISTAMINE-INDUCED NASAL BLOCKAGE

### 3.1 Introduction

Histamine has long been associated with allergic diseases and anaphylaxis (Dale HH, 1929; Dragstedt CA *et al.*, 1932; Bovet D, 1950). Allergic rhinitis is defined as the abnormal inflammation of the lining of the nose provoked by an allergen and so is categorised as an allergic disease. Furthermore, histamine has been shown to be released from nasal mast cells (Howarth PH, 1995; Otsuka H *et al.*, 1995), and histamine levels in nasal lavages increase following allergen challenge (Naclerio RM *et al.*, 1985; Walden SM *et al.*, 1988).

Since the development of selective H<sub>2</sub> antagonists (Black JW *et al.*, 1972), histamine receptor heterogeneity was thought to explain the different endogenous actions of histamine: H<sub>1</sub>-mediation of vascular and neuronal pathways and H<sub>2</sub>-control of gastric acid secretion. The subsequent discovery of H<sub>3</sub> and H<sub>4</sub> receptors complicated this classification of the roles of endogenous histamine and now histamine receptors other than H<sub>1</sub> have been suggested to play a role in histamine-induced inflammation (Howarth PH *et al.*, 2000). In this chapter the contribution of H<sub>1</sub> receptors to the nasal symptoms, and particularly the nasal blockage, caused by histamine after it has been sprayed into the nasal cavities of normal, non-atopic, human subjects, has been investigated.

The two H<sub>1</sub> receptor antagonists used in this chapter, chlorpheniramine and cetirizine, are both highly selective for H<sub>1</sub> receptors as compared to H<sub>2</sub>, H<sub>3</sub>, serotonergic, adrenergic and muscarinic receptors (Bernheim J *et al.*, 1991; Kato M *et al.*, 1997; Gillard M *et al.*, 2003); with the exception of the minor anti-muscarinic properties of chlorpheniramine (Fang SY *et al.*, 1998).



## 3.2 Experimental protocol

In the following double-blind experiments, healthy non-atopic human volunteers were used as subjects.

### 3.2.1 The effect of topical histamine on nasal patency

The aim of this experiment was to assess the effect of histamine on nasal symptoms, as recorded by sneeze counts, subjective scores for rhinorrhea and congestion, and acoustic rhinometry (objective measurement of nasal patency). Subjects received, by 100µl aerosol into each nostril, either saline or histamine, 40, 100 or 400µg. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry, visual analog scores (VAS) (for congestion and rhinorrhea) were completed by the subjects and any sneezes were counted. 6 subjects were included in this study. The doses and time-course used were based upon pilot studies and previous publications (Turner PJ *et al.*, 2000b; Austin CE *et al.*, 1994c). The protocol is summarized in Figure 3.1.

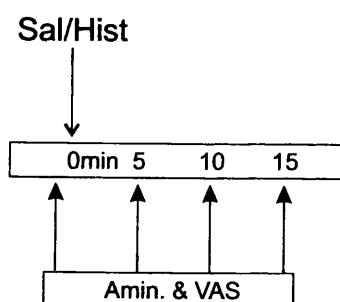


Figure 3.1: Protocol for investigating the effect of histamine on nasal patency. Sal = saline, Hist = histamine.

### 3.2.2 The effect of unilateral histamine challenge on nasal patency

The aim of this experiment was to investigate the effect of unilateral histamine challenge on the nasal patency in both the ipsilateral and contralateral nasal cavities, as measured by acoustic rhinometry. Subjects received, by 100µl aerosol, histamine, 400µg, into a single nostril (no aerosol was sprayed into the other

nostril). Each subject was challenged in each nostril, in addition to a non-challenge test (no spray into either nostril), on separate days, in random order. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 3.2.

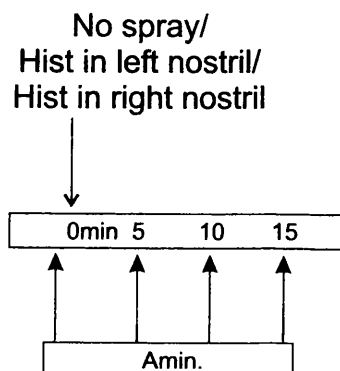


Figure 3.2: Protocol for investigating the effect of unilateral histamine challenge on nasal patency. Hist = histamine.

### 3.2.3 The effect of topical histamine H<sub>1</sub> antagonist, chlorpheniramine, on histamine induced nasal blockage

The aim of this experiment was to investigate the antagonism of histamine-induced nasal blockage by a topical H<sub>1</sub> antagonist, as measured by acoustic rhinometry. Subjects received, by 100µl aerosol into each nostril, either saline or chlorpheniramine, 1mg. 5 minutes later, subjects received, by 100µl aerosol into each nostril, either saline or histamine, 400µg. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 6 subjects were included in this study. The protocol is summarized in Figure 3.3.

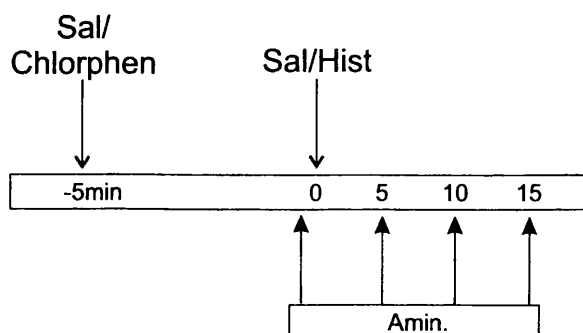


Figure 3.3: Protocol for investigating the effect of topical chlorpheniramine on histamine-induced nasal blockage. Sal = saline, Chlorphen = chlorpheniramine, Hist = histamine.

### 3.2.4 The effect of oral histamine H<sub>1</sub> antagonists on histamine-induced nasal blockage

The aim of this experiment was to investigate the degree to which H<sub>1</sub> antagonists could inhibit histamine-induced nasal blockage, as measured by acoustic rhinometry. Subjects received oral chlorpheniramine, 2, 8 or 16mg, or oral cetirizine, 5, 20, or 30mg or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 8 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Chlorpheniramine, 2mg; histamine, 400µg
- iv) Chlorpheniramine, 8mg; histamine, 400µg
- v) Chlorpheniramine, 16mg; histamine, 400µg
- vi) Cetirizine, 5mg; histamine, 400µg
- vii) Cetirizine, 20mg; histamine, 400µg
- viii) Cetirizine, 30mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The doses and time-course (pretreatment time and interval between separate treatments) of this study was based on the published pharmacokinetics (Huang SM *et al.*, 1982; DuBuske L, 1995; Urien S *et al.*, 1999). The protocol is summarized in Figure 3.4.

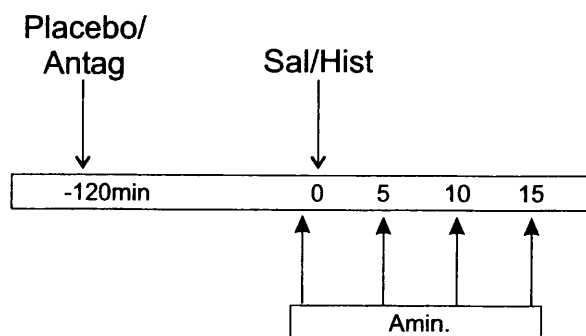


Figure 3.4: Protocol for investigating the effect of oral H<sub>1</sub> antagonists on histamine-induced nasal blockage. Antag = H<sub>1</sub> antagonist, Sal = saline, Hist = histamine.

### 3.2.5 The effect of high doses of oral histamine H<sub>1</sub> antagonist, cetirizine, on histamine-induced nasal blockage

The aim of this experiment was to investigate whether or not a maximal inhibition of histamine-induced nasal blockage could be achieved with an H<sub>1</sub> antagonist; and, in addition, to assess if there was any residual histamine-induced nasal blockage in the presence of that dose of H<sub>1</sub> antagonist. Subjects received oral cetirizine, 20, 25, 30 or 35mg, or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received each treatment in a random order, with at least 48 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 3.5.

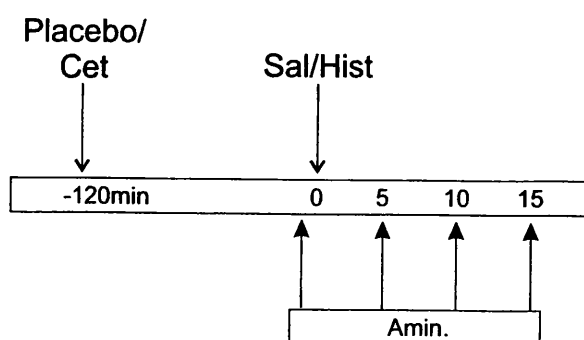


Figure 3.5: Protocol for investigating the effect of high dose cetirizine on histamine-induced nasal blockage. Cet = cetirizine, Sal = saline, Hist = histamine.

### 3.2.6 The effect of pH on nasal saline challenge

The aim of this experiment was to investigate any changes in nasal patency as a result of the increase in aerosol acidity caused by the dissolved histamine salt. 3 separate solutions of 4mg/ml histamine, dissolved in saline, were analysed with a pH meter. The mean pH of 4mg/ml histamine was calculated as 4.10 ( $\pm$  0.05). 3 separate solutions of saline were also analysed with a pH meter. The mean pH of saline was calculated as 4.63 ( $\pm$  0.06).

A stock solution of saline was adjusted, using 0.05mM HCl, to pH 4.10.

Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline, pH 4.10, or saline, pH 4.63. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 3.6.

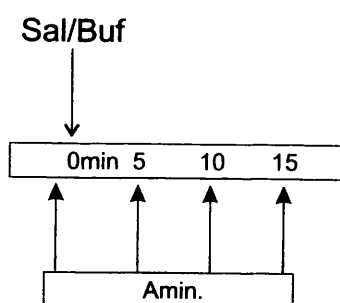


Figure 3.6: Protocol for investigating the effect of pH on saline nasal challenge as measured by acoustic rhinometry. Sal = saline, pH 4.63, Buf = buffered saline, pH 4.10.

### 3.2.7 Data analysis

Nasal patency was measured using acoustic rhinometry, as described in Chapter 2. Amin. was recorded immediately prior to and 5, 10 and 15 minutes after nasal challenge. Amin. values were normalised to the Amin. value recorded prior to nasal challenge. For each challenge, a normalised Amin. versus time curve was plotted, and the area under the curve (AUC) calculated. Subjective symptom scores of congestion and rhinorrhea were assessed using visual analog scores (VAS), as

described in Chapter 2. Post-challenge VAS were corrected for any recorded pre-challenge value by subtracting the pre-challenge value from the recordings made following nasal challenge. For each challenge, a corrected VAS versus time curve was plotted, and the area under the curve calculated. Thus the presented VAS data represents only the total change in symptom scores over a 15 minute period following challenge. The rhinometry and VAS data are presented either as mean values, together with s.e.mean; or as individual data. The appropriate non-parametric statistical test is given with each data set.  $p < 0.05$  is taken as significant.

### 3.3 Results

#### 3.3.1 The effect of topical histamine on nasal patency

Application, by aerosol into each nostril, of histamine, 100 and 400µg, caused a significant reduction in AUC (area under the normalised Amin. versus time curve) as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge, as shown in Figure 3.7.

Application, by aerosol into each nostril, of histamine, 40 to 400µg, caused a significant increase in the number of sneezes counted as compared to saline over the 15 minutes after nasal challenge ( $p < 0.05$ , Wilcoxon sign-rank test), as shown in Figure 3.8.

Application, by aerosol into each nostril, of histamine, 40 to 400µg, caused a significant increase in visual analog scores for both congestion and rhinorrhea as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased congestion and rhinorrhea experienced by the subjects over the 15 minutes after nasal challenge, as shown in Figure 3.9.

#### 3.3.2 The effect of unilateral histamine challenge on nasal patency

Unilateral nasal challenge, by aerosol, of histamine, 400µg, caused a significant reduction in AUC in the ipsilateral nasal cavity as compared to no aerosol challenge ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Unilateral histamine challenge had no effect on the contralateral nasal cavity ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 3.10).

#### 3.3.3 The effect of topical histamine H<sub>1</sub> antagonist, chlorpheniramine, on histamine induced nasal blockage

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test),

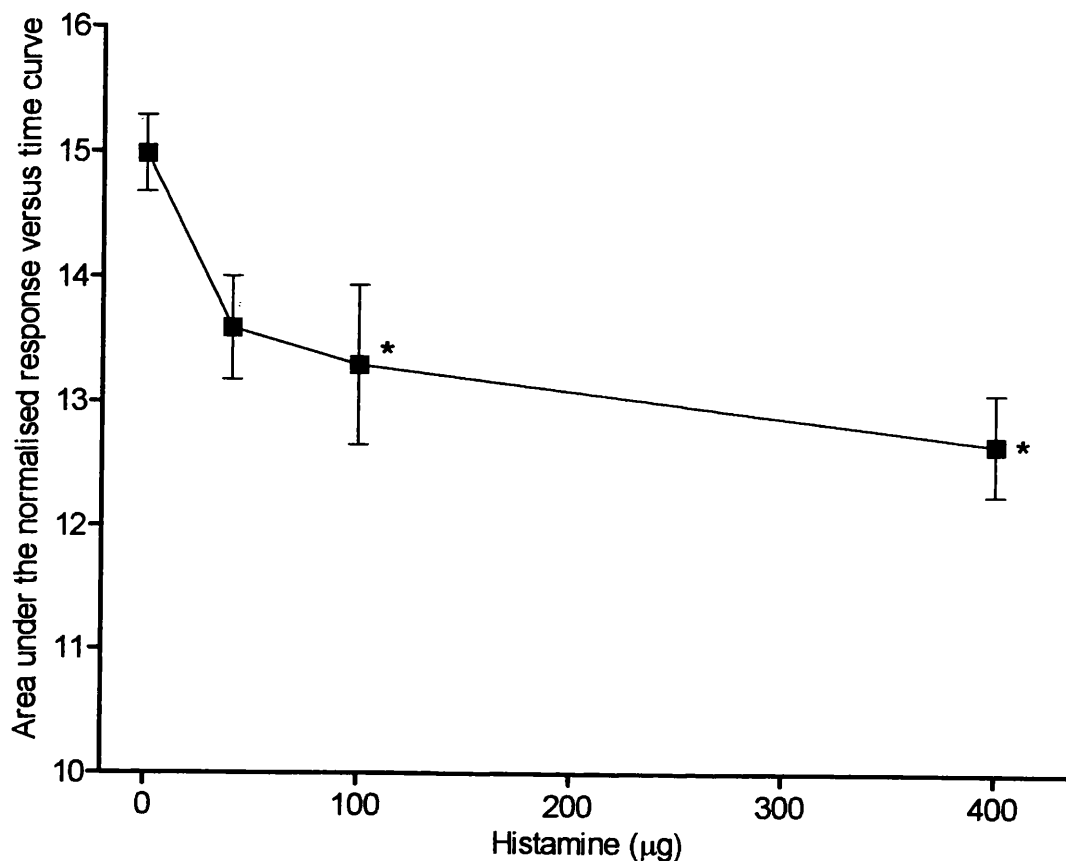


Figure 3.7: Dose-response curve for the action of histamine on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of histamine as an aerosol, at the dose shown, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.659cm<sup>2</sup>, s.e.mean of 0.051cm<sup>2</sup>). Data are means from 6 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control (p<0.05, Wilcoxon sign-rank test).



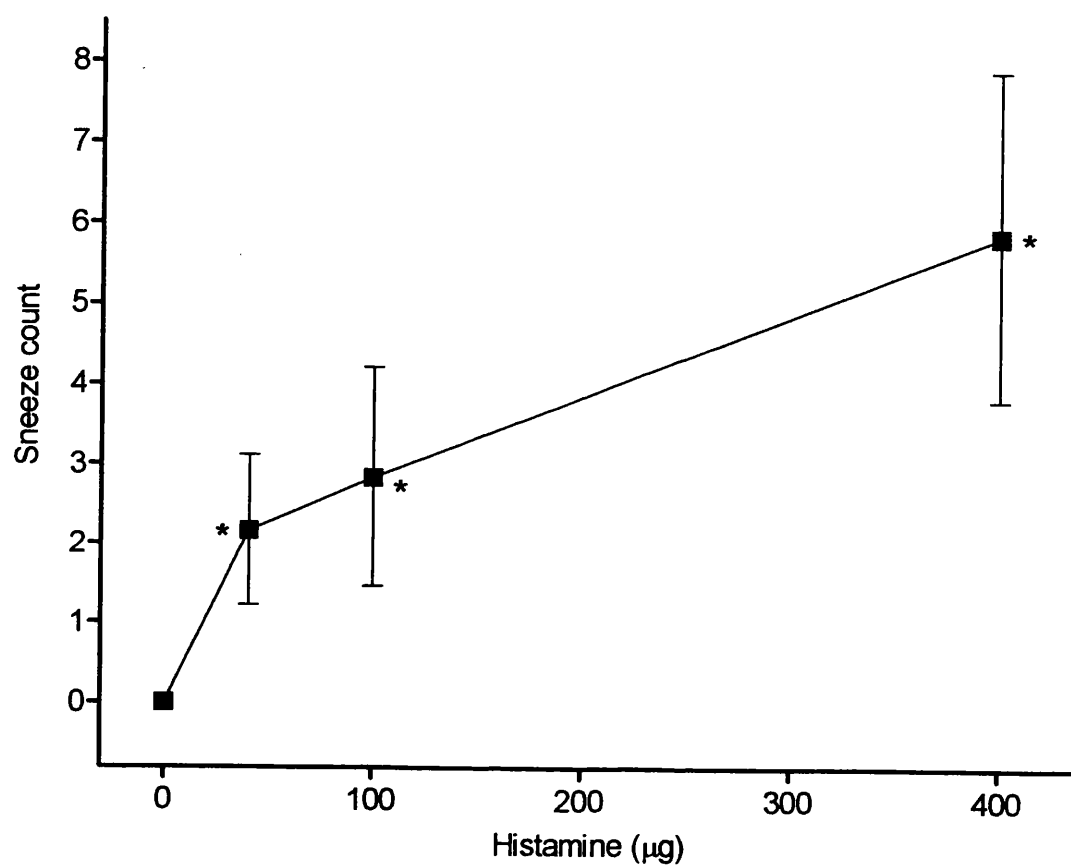


Figure 3.8: Dose-response curve for the action of histamine on the number of sneezes measured over a period of 15 minutes following the administration of histamine as an aerosol, at the dose shown, into each nostril. Data are means from 6 people. Vertical bars represent s.e.mean. \* Significant increase in the number of sneezes as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test).

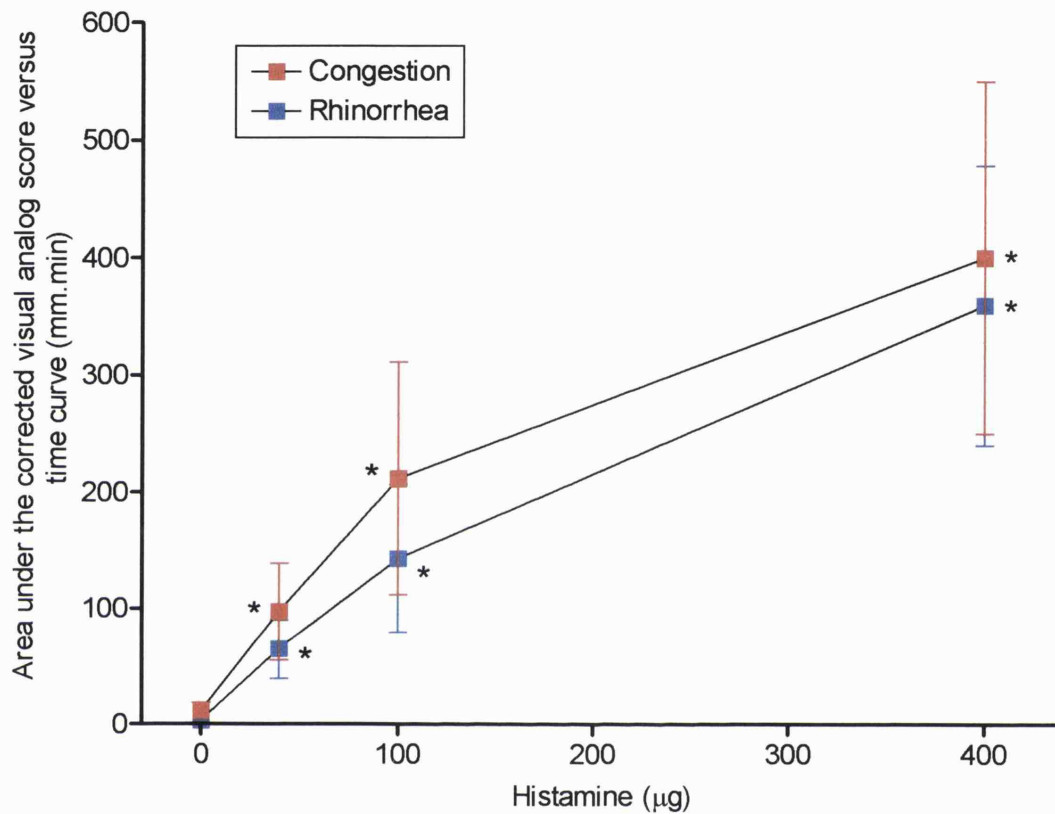


Figure 3.9: Dose-response curve for the action of histamine on the area under the corrected visual analog score for congestion (■) and rhinorrhea (■) measured over a period of 15 minutes following the administration of histamine as an aerosol, at the dose shown, into each nostril. Visual analog scores for both congestion and rhinorrhea were measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Visual analog scores were corrected for any measured pre-challenge value by subtracting the pre-challenge response (mm) from the recorded post-challenge responses (mm). The corrected VAS were then plotted against time and the area under the curve calculated. Data are means from 6 people. Vertical bars represent s.e.mean. \* Significant increase in visual analog scores as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test).

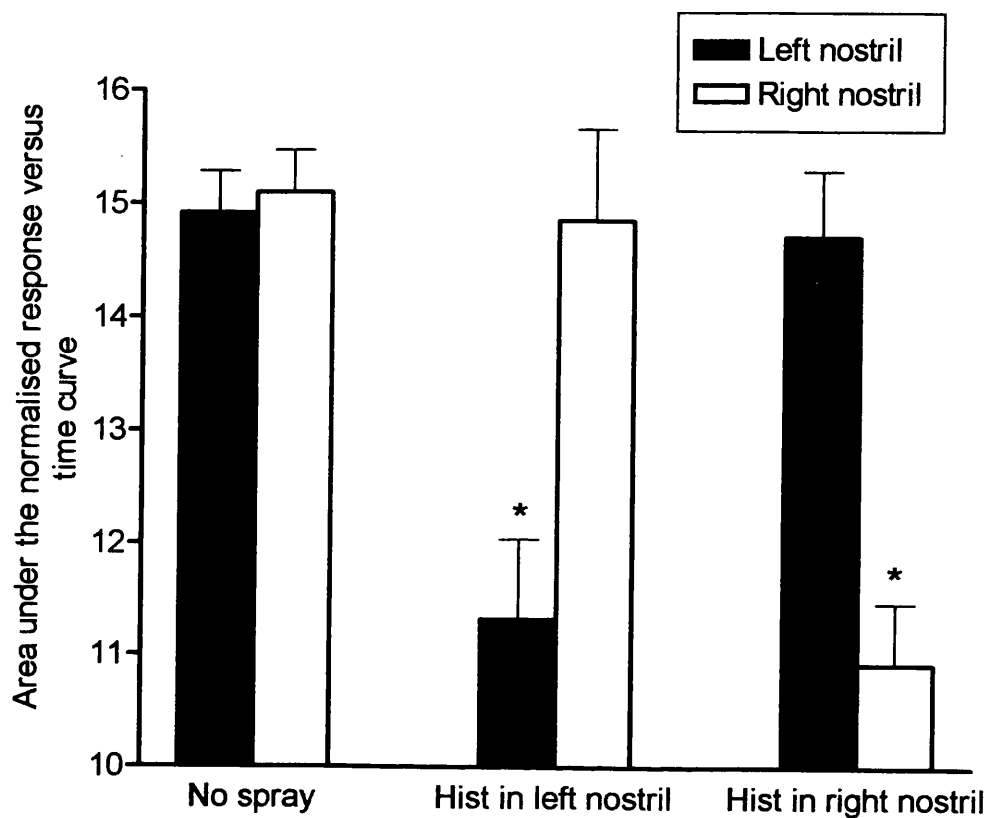


Figure 3.10: The effect of unilateral histamine challenge on the area under the Amin. versus time curve measured over a period of 15 minutes for each nostril following administration of histamine, 400µg, as an aerosol into a single nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Nostril Amin. values were not combined in an overall nasal Amin. value, but were instead kept separate and were normalised to the pre-challenge value of the given nostril (Left mean of 0.683cm<sup>2</sup>, s.e.mean of 0.054cm<sup>2</sup>; Right mean of 0.609cm<sup>2</sup>, s.e.mean of 0.028cm<sup>2</sup>). Data are means from 8 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to control ( $p < 0.05$ , Wilcoxon sign-rank test).

corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment of the nasal cavity with chlorpheniramine, 1mg, by aerosol into each nostril, 5 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 3.11). Pretreatment of the nasal cavity with chlorpheniramine, 1mg, had no effect on saline nasal challenge ( $p > 0.05$ , Wilcoxon sign-rank test).

### **3.3.4 The effect of oral histamine H<sub>1</sub> antagonists on histamine-induced nasal blockage**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge.

Pretreatment with oral chlorpheniramine, 8 and 16mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 3.12).

Pretreatment with oral cetirizine, 20 and 30mg, 2 hours prior to nasal challenge, also significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test), although, again, the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 3.13).

### **3.3.5 The effect of high doses of oral histamine H<sub>1</sub> antagonist, cetirizine, on histamine-induced nasal blockage**

Increasing the dose of oral cetirizine, given 2 hours prior to nasal challenge, from 20mg through to 35mg, had no effect on saline nasal challenge ( $p > 0.05$ , Friedman's test). Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline when pretreated with 25mg or 35mg cetirizine ( $p < 0.05$ , Wilcoxon sign-rank test). However, application, by aerosol

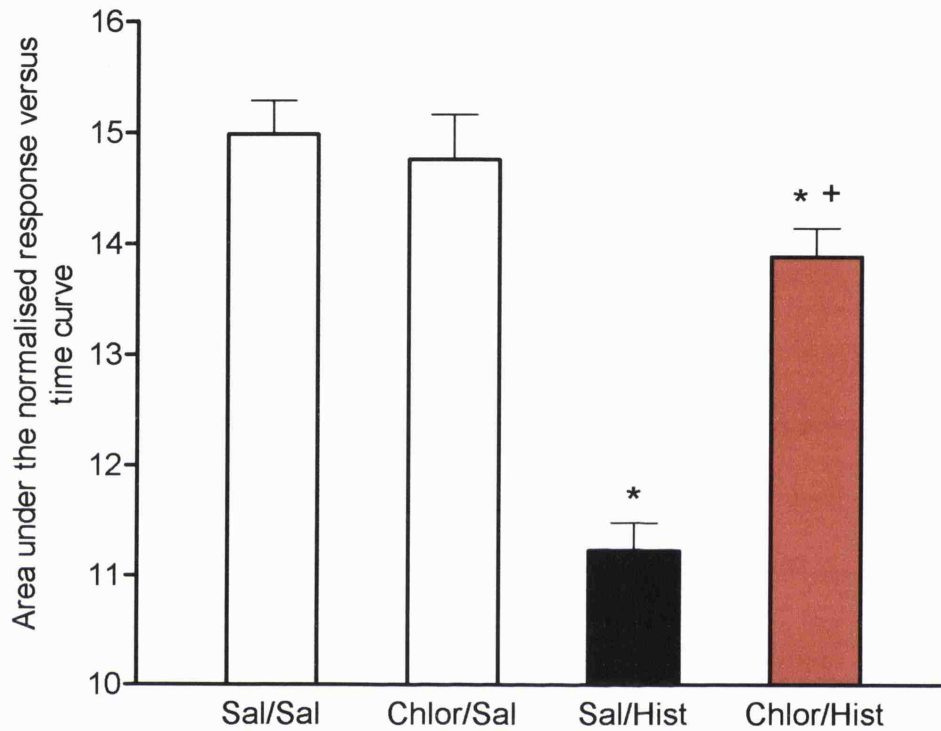


Figure 3.11: The effect of chlorpheniramine [Chlor], 1mg, administered as an aerosol into each nostril 5 minutes prior to challenge with histamine, on the nasal blockage caused by histamine [Hist], 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each saline/histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.710cm<sup>2</sup>, s.e.mean of 0.059cm<sup>2</sup>). The data are the means from 6 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline [Sal] control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without chlorpheniramine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

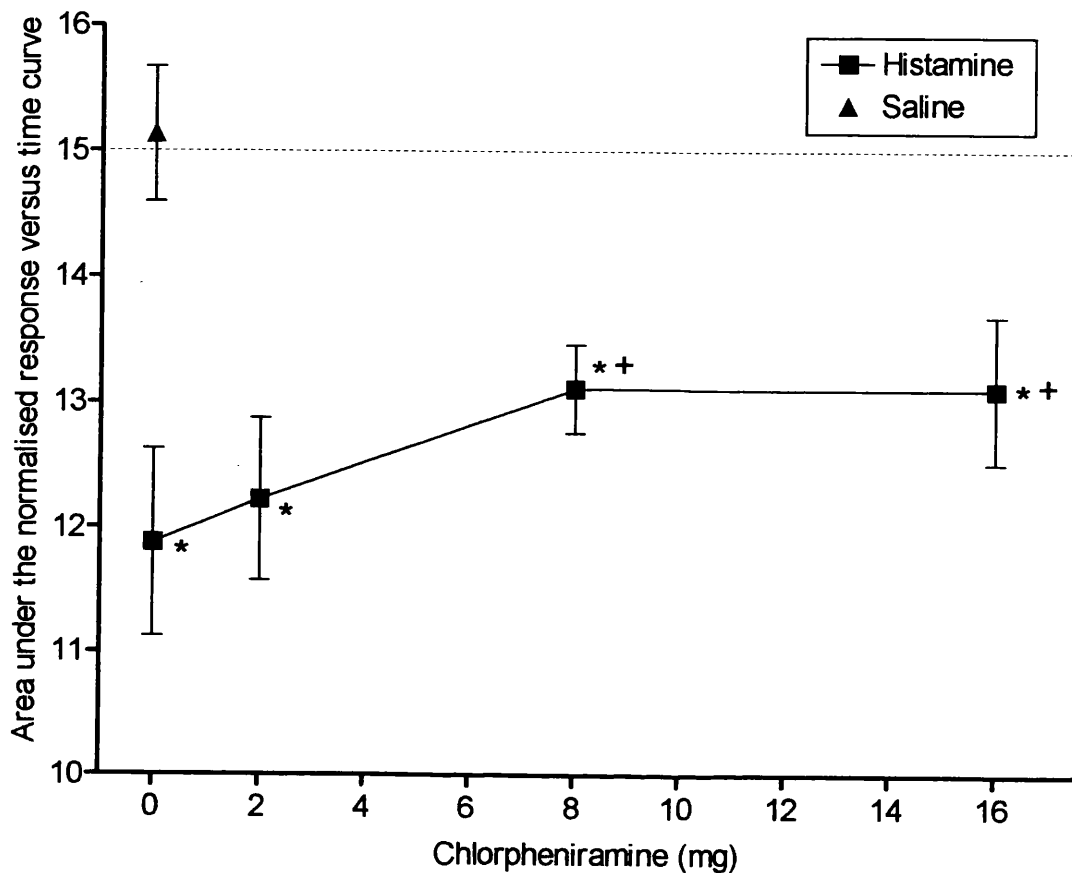


Figure 3.12: Dose-response curve for the inhibition by orally administered chlorpheniramine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.629cm<sup>2</sup>, s.e.mean of 0.015cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by  $H_1$  antagonists (Figure 3.11) (Kirkegaard J *et al.*, 1983; Austin CE *et al.*, 1996c). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without chlorpheniramine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

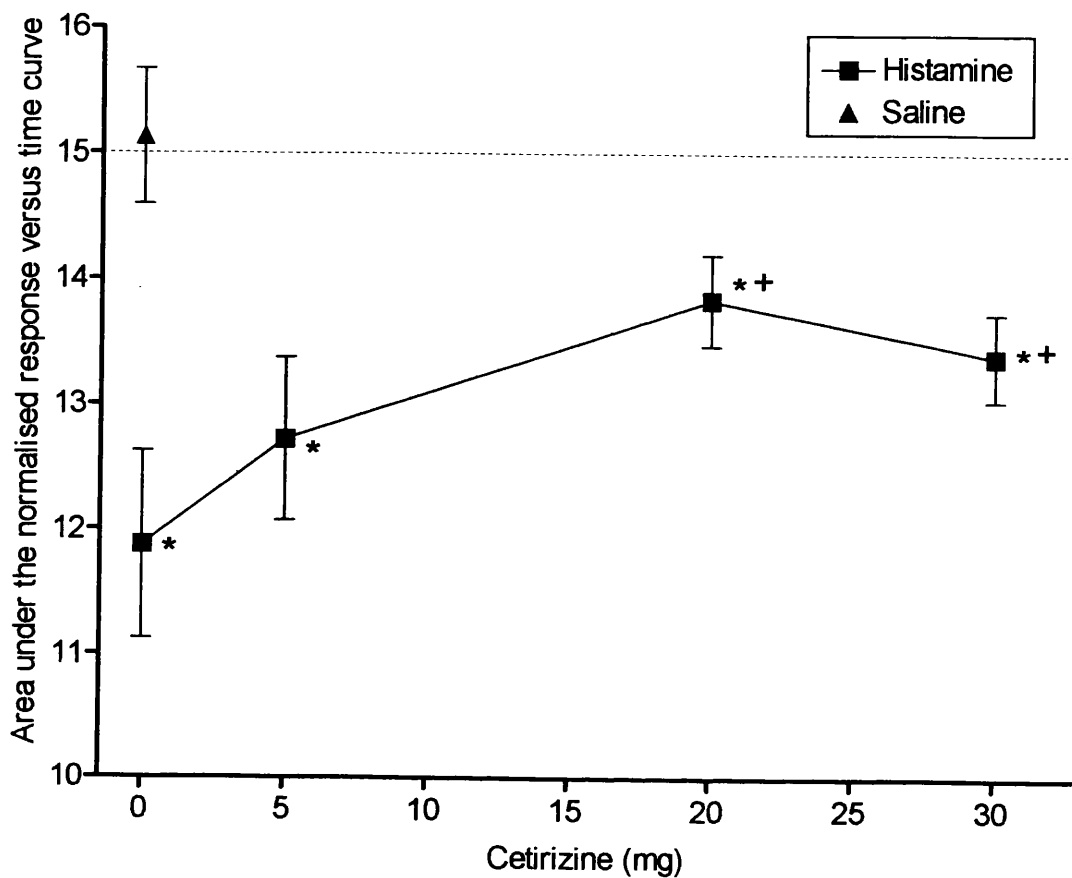


Figure 3.13: Dose-response curve for the inhibition by orally administered cetirizine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.629cm<sup>2</sup>, s.e.mean of 0.015cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by H<sub>1</sub> antagonists (Figure 3.11) (Kirkegaard J *et al.*, 1983; Austin CE *et al.*, 1996c). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

to each nostril, of histamine, 400µg, did not caused a significant change in AUC when pretreated with 20mg or 30mg cetirizine ( $p < 0.05$ , Wilcoxon sign-rank test). Increasing the dose of oral cetirizine, given 2 hours prior to nasal challenge, from 20mg through to 35mg, had no effect on histamine nasal challenge ( $p > 0.05$ , Friedman's test) (Figure 3.14).

Both histamine and saline nasal challenges were not significantly altered by increasing the cetirizine dose above 20mg ( $p > 0.05$ , Friedman's test), which was suggested from the previous study (3.3.4) which showed that neither cetirizine nor chlorpheniramine, at maximal doses, abolished the histamine reduction in AUC. Given this, the AUC (area under the normalised Amin. versus time curve) data for saline nasal challenge at each cetirizine dose (Table 3.1) was combined into a single value for each subject. This value was reached by first plotting AUC against cetirizine dose, and then calculating the integral of AUC and cetirizine dose ( $\int_{35}^{20} \text{AUC} \delta \text{cetirizine dose}$ ) for each subject (see insert, Figure 3.15). This value then represented the total effect of saline, following pretreatment of cetirizine, 20mg through to 35mg, on the normalised Amin. over a 15 minute period after nasal challenge. The units of this value are unit.min.mg. This calculation was repeated with the histamine AUC data for each subject (Table 3.1). The total effect of saline and histamine, following pretreatment of cetirizine, 20mg through to 35mg, on the normalised Amin. over a 15 minute period after nasal challenge is shown in Figure 3.15.

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in the area under the AUC versus cetirizine dose curve as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to significant histamine-induced nasal blockage in the presence of maximal doses of cetirizine, as shown in Figure 3.15. Thus, cetirizine appeared not to be able totally to inhibit nasal blockage caused by histamine. In the presence of cetirizine at high doses there is residual nasal blockage.

### 3.3.6 The effect of pH on nasal saline challenge

Application, by aerosol to each nostril, of buffered saline (corrected to pH 4.10) had no effect on AUC as compared to normal saline (pH 4.63), corresponding to no



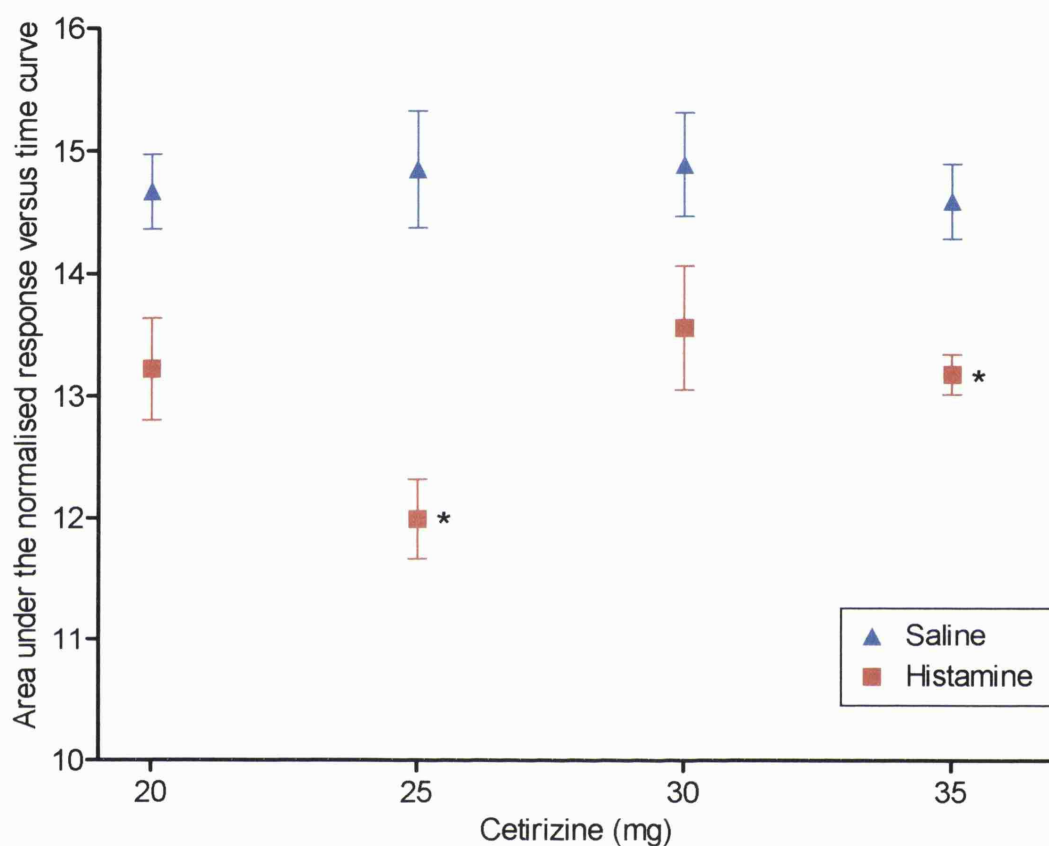


Figure 3.14: Dose-response curve for the inhibition by orally administered cetirizine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.672cm<sup>2</sup>, s.e.mean of 0.028cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

	Saline nasal challenge				Histamine nasal challenge			
	Cetirizine (mg)				Cetirizine (mg)			
	20	25	30	35	20	25	30	35
Subject 1	14.98	17.26	14.18	15.14	12.72	12.36	14.51	13.58
Subject 2	14.15	14.16	14.02	12.72	12.12	10.64	11.71	13.89
Subject 3	14.92	15.01	16.59	14.85	15	11.2	13.93	13
Subject 4	16.08	14.17	14.49	14.01	12.86	12.96	14.31	12.47
Subject 5	15.29	16.55	14.97	15.37	12.81	11.68	15.76	12.77
Subject 6	14.57	14.32	13.28	14.92	14.56	12.82	13.9	13.4
Subject 7	14.18	13.83	14.93	14.58	14.01	13.05	12.64	12.99
Subject 8	13.21	13.52	16.64	15.13	11.7	11.25	11.69	13.34

Table 3.1: Dose-response curve for the inhibition by orally administered cetirizine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400µg, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.672cm<sup>2</sup>, s.e.mean of 0.028cm<sup>2</sup>). The data values are the responses from 8 individuals and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of saline/histamine.

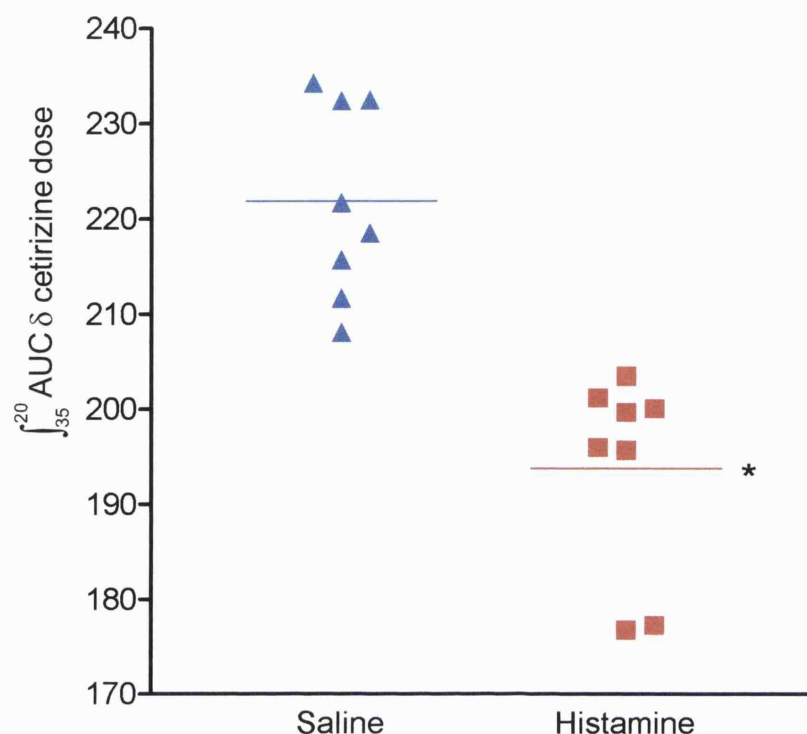
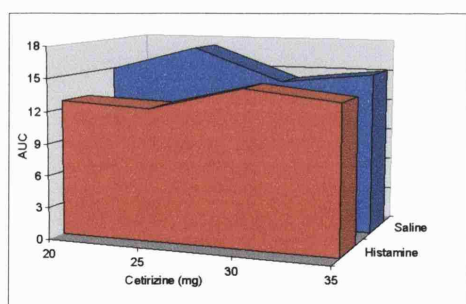


Figure 3.15: The residual effect of histamine, 400µg, administered as an aerosol into each nostril, on nasal patency in the presence of maximal doses of cetirizine (20, 25, 30 and 35mg), given 2 hours prior to nasal challenge. The response [AUC] of each individual to histamine in the presence of 4 doses of cetirizine (20, 25, 30 or 35mg) (Table 3.1) was plotted against cetirizine dose (see insert for example using Subject 1 data) and the integral



( $\int_{35}^{20} \text{AUC } \delta \text{ cetirizine dose}$ ) was calculated (200.1 unit.min.mg for Subject 1). Thus the responses for histamine (■) following pretreatment with the 4 doses of cetirizine were pooled into one value for each individual that corresponds to the total effect of histamine in the presence of cetirizine, 20mg through to 35mg. In

addition, the response [AUC] of each individual to saline in the presence of 4 doses of cetirizine (20, 25, 30 or 35mg) (Table 3.1) was plotted against cetirizine dose (see insert for example using Subject 1 data) and the integral ( $\int_{35}^{20} \text{AUC } \delta \text{ cetirizine dose}$ ) was calculated (232.5 unit.min.mg for Subject 1). Thus the responses for saline (▲) following pretreatment with the 4 doses of cetirizine were also pooled into one value for each individual that corresponds to the total effect of saline in the presence of cetirizine, 20mg through to 35mg. The data in Figure 3.15 are the calculated responses from 8 individuals and represent the integral of AUC (area under the normalised  $A_{\text{min}}$  versus time curve

measured over a 15 minute period following nasal challenge) and cetirizine dose:

$\int_{35}^{20} \text{AUC } \delta \text{cetirizine dose}$ . Horizontal bars represent the mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

significant change in nasal patency over the 15 minutes after nasal challenge ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 3.16).

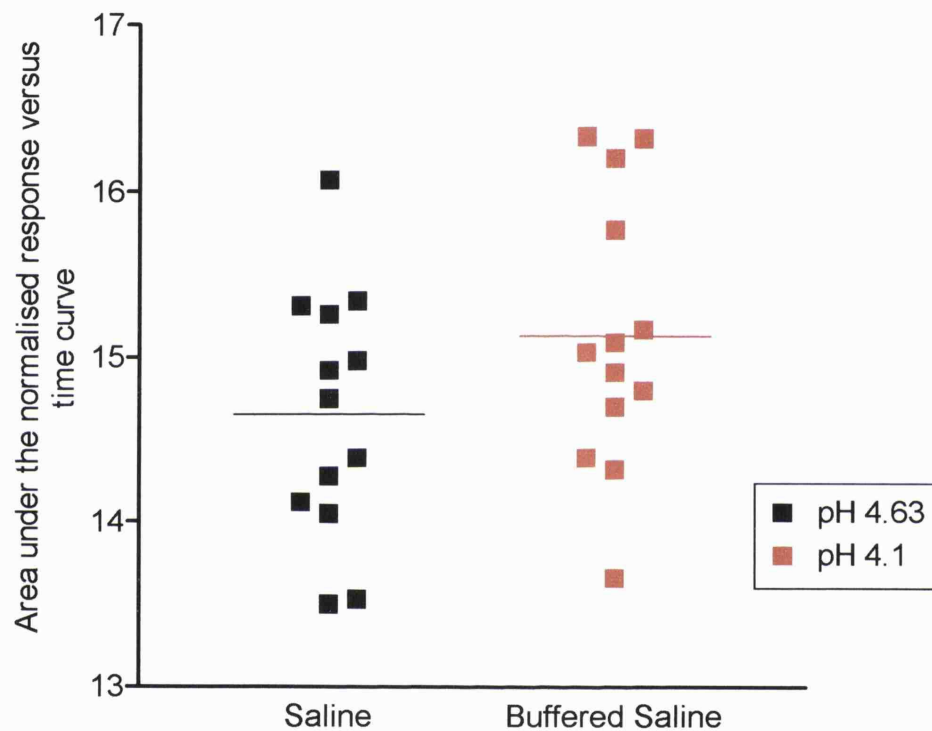


Figure 3.16: The effect of pH change on the area under the Amin. versus time curve measured over a period of 15 minutes following administration of saline as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of  $0.692\text{cm}^2$ , s.e.mean of  $0.044\text{cm}^2$ ). The data points are the individual responses from 8 subjects. Horizontal bars represent the mean.

### 3.4 Discussion

Histamine, following bilateral nasal administration as an aerosol, 40µg to 400µg, caused an increase in nasal symptoms. Visual analog scores of congestion and rhinorrhea showed a significant increase with increasing doses of histamine (40µg to 400µg). The number of sneezes counted also significantly increased with increasing doses of histamine (40µg to 400µg). Acoustic rhinometry measurements showed a significant reduction in the area under the normalised  $A_{min}$  versus time curve measured over a period of 15 minutes (AUC) following nasal administration of histamine, 100 and 400µg. This reduction in AUC represents an objectively measured reduction in nasal patency or, alternatively, increased nasal blockage. The nasal blockage is thought to be due to an overall vasodilation of the nasal arterioles and capacitance vessels, not due to nasal mucosal oedema, because histamine-induced nasal blockage is reversed by sympathomimetics (which do not reverse oedema) (Cole P *et al.*, 1983). These histamine-induced nasal symptoms are in agreement with previous studies which showed that histamine, at similar doses, causes an increase in rhinorrhea, sneezing and nasal blockage (measured both subjectively and objectively) (Miadonna A *et al.*, 1987; Mullins RJ *et al.*, 1989; Doyle WJ *et al.*, 1990; Rajakulasingam K *et al.*, 1993; Austin CE *et al.*, 1994c; Birchall MA *et al.*, 1996; Turner PJ *et al.*, 2000b).

400µg histamine, at the top of the dose-response curve (Figure 3.7), was considered to be the best dose to use in order to investigate the role of histamine in the control of nasal patency further. 400µg histamine caused a substantial reduction in AUC but was not considered too unpleasant for further experiments.

Unilateral histamine challenge (400µg) caused a significant reduction in AUC in the ipsilateral nasal cavity. This local effect was in agreement with studies which showed that histamine exerts a topical effect *in vivo* in skin (Davies MG *et al.*, 1980) and in the nose (Kirkegaard J *et al.*, 1983); and in *in vitro* bronchial (Ramsay SG *et al.*, 1997) and gut (Callaway JK *et al.*, 1990) preparations. Furthermore, a study of the inhibition of some of the effects of histamine by topical chlorpheniramine (H<sub>1</sub> antagonist), showed that the antagonist was only effective when sprayed in the same nasal cavity as the histamine (Kirkegaard J *et al.*, 1983), indicating that at least one histamine receptor mechanism for causing inflammation was present in

the nasal mucosa. Thus histamine exerts at least some of its actions locally and so it is not surprising that histamine caused significant ipsilateral nasal blockage.

No contralateral changes in nasal patency were recorded following unilateral histamine challenge (400µg). As discussed in Chapter 1.6.2, there is little agreement in the literature concerning contralateral changes in nasal patency following unilateral histamine challenge: one study reported a decrease in nasal patency (Shelton D *et al.*, 1994), another study showed no change in patency (Kirkegaard J *et al.*, 1983) and a further study reported an increase in nasal patency (Birchall MA *et al.*, 1993).

An increase in contralateral nasal patency could be explained by a central reflex increase in sympathetic outflow: activation of ipsilateral C fibres by histamine would cause action potential propagation up to the trigeminal spinal tract where interneurons would stimulate an increase in sympathetic excitation, resulting in an increase in noradrenaline (NA) release from both ipsilateral and contralateral sympathetic neurones, thus promoting an increase in nasal patency. Although there is little by way of evidence for this in the literature, histamine- and allergen-induced activation of a central reflex-mediated increase in parasympathetic activity is well documented (Baraniuk JN, 1992; Baroody FM *et al.*, 1994; Birchall MA *et al.*, 1996; Georgitis JW, 1998). If histamine does stimulate a central reflex-mediated increase in sympathetic outflow then there would be an even greater decongestant effect for the histamine to overcome in order to cause nasal blockage in the ipsilateral cavity. In this way the actual vasodilatory (and thus nasal blockage) effect of histamine would be greater than the effect measured by acoustic rhinometry, as histamine would be functionally antagonized by an increased level of noradrenaline.

However, in this study, there was no increase in contralateral nasal patency, thus there was no evidence of this hypothetical central reflex-mediated increase in sympathetic outflow. This may be because there is no increase in sympathetic activity or the increase in decongestive NA is balanced by an increase in contralateral vasodilation. Three possible explanations for an increase in contralateral vasodilation are (i) histamine diffuses across the septum into the contralateral cavity; (ii) the blood vessels of both cavities are directly connected;



and (iii) a central reflex causes the release of vasodilatory mediators from contralateral parasympathetic neurones.

No studies investigating the diffusion of histamine (or similar substances) from one nasal cavity to the other can be found in the literature. Although exogenous histamine may well diffuse through the nasal mucosa (sneezing can occur within 5 seconds of histamine nasal challenge), the distance to the neighbouring cavity and the cartilaginous septum is likely to be a significant barrier to the diffusion of the positively charged histamine. Alternatively, the histamine aerosol may reach far enough up the nasal airway to gain access to the contralateral cavity via the nasopharynx (the area posterior to the cavities where they join together).

Blood vessels connecting one cavity to the other have been identified in dogs, pigs and cats (Lung MA *et al.*, 1984). However, these collateral anastomoses have not been found in man and so it seems unlikely that histamine in the blood vessels of the ipsilateral cavity would circulate to any significant degree to the other cavity.

The contribution of the parasympathetic to the control of nasal patency has been discussed in Chapter 1.7.2. Histamine can cause the release of the vasodilators, ACh and VIP (Kalinin MA, 1994). Both methacholine and VIP have been shown to cause nasal blockage in man (Devillier P *et al.*, 1988; Chatelain C *et al.*, 1995), although methacholine has also shown to have no effect on nasal patency (Zanoletti E *et al.*, 1999). In addition, oxitropium, a muscarinic antagonist, reduces histamine-induced rhinorrhea (Birchall MA *et al.*, 1996). In the same study, the oxitropium also reduced the histamine-induced nasal blockage. Unfortunately, this study is the only publication using muscarinic antagonists to inhibit the nasal actions of histamine, thus it is difficult to assess the relevance of the parasympathetic to histamine-induced nasal blockage. Although the contribution of histamine to allergen-induced symptoms has not been clarified, it is important to remember that muscarinic antagonists have been widely shown to have no effect on allergen-induced nasal blockage (Mygind N, 1982; Baroody FM *et al.*, 1994).

Oral cetirizine and nasal administration of chlorpheniramine (both H<sub>1</sub> antagonists) had no effect on saline nasal challenge, indicating that H<sub>1</sub> antagonists have no effect on resting nasal patency, which is in agreement with the available literature

(Kirkegaard J *et al.*, 1983; Havas TE *et al.*, 1986; Hilberg O *et al.*, 1995; Austin CE *et al.*, 1996; Wood-Baker R *et al.*, 1996).

Pretreatment with chlorpheniramine (either nasally or orally) or oral cetirizine significantly inhibited the histamine-induced nasal blockage, indicating that histamine causes nasal blockage via H<sub>1</sub> receptors. The data is in accord with a study which showed that the selective H<sub>1</sub> agonist, betahistine, also causes nasal blockage (Shelton D *et al.*, 1994). H<sub>1</sub>-mediated nasal blockage has been frequently shown by studies inhibiting histamine-induced nasal blockage with topical and systemic H<sub>1</sub> antagonists (Secher C *et al.*, 1982; Kirkegaard J *et al.*, 1983; Mygind N *et al.*, 1983; Havas TE *et al.*, 1986; Hilberg O *et al.*, 1995; Wang DY *et al.*, 2001). However, another study showed no significant reduction of histamine-induced nasal blockage by oral cetirizine (10mg) (Wood-Baker R *et al.*, 1996). That topical chlorpheniramine inhibited histamine-induced nasal blockage when nasally administered 5 minutes prior to histamine challenge suggests that the H<sub>1</sub> receptors involved in the nasal blockage are located within the nasal mucosa. In addition, cetirizine, the second generation H<sub>1</sub> antagonist which is unable to cross the blood brain barrier (Campoli-Richards DM *et al.*, 1990), reduced histamine-induced blockage, indicating that these H<sub>1</sub> receptors are not located in the CNS.

H<sub>1</sub> receptors have been shown, using autoradiography, on human inferior turbinate vascular endothelium (Ishibe T *et al.*, 1985; Okayama M *et al.*, 1992) and H<sub>1</sub> receptor mRNA has also been shown in human nasal turbinates (Iriyoshi N *et al.*, 1996). Histamine causes H<sub>1</sub> antagonist-sensitive vasodilation in many tissues, such as rat basilar arteries (Chang JY *et al.*, 1988), guinea-pig cutaneous arteries (Owen DA *et al.*, 1980), dog hindlimb blood vessels (Powell JR *et al.*, 1976b), rat mesenteric arteries (Yousif MH *et al.*, 2002), monkey basilar arteries (Fujiwara M *et al.*, 1992), guinea-pig nasal blood vessels (Bockman CS *et al.*, 2002), pig nasal blood vessels (Alving K *et al.*, 1991; Malis DD *et al.*, 2001), human coronary arteries (Toda N *et al.*, 1989), human pulmonary arteries (Ortiz JL *et al.*, 1992) and human nasal blood vessels (Birchall MA *et al.*, 1993). In the *in vitro* studies where the endothelium was removed, the histamine-induced vasodilation was reduced and its sensitivity to H<sub>1</sub> antagonism was eliminated (Chang JY *et al.*, 1988; Ortiz JL *et al.*, 1992), and in one study endothelium denudation unmasked a minor H<sub>1</sub>-mediated vasoconstriction (Toda N *et al.*, 1989). Thus the H<sub>1</sub>-mediated vasodilation appears

to be endothelium-dependent and, perhaps, there is a separate H<sub>1</sub> receptor population on vascular smooth muscle cells which mediates vasoconstriction (although there is no evidence for this in human nasal blood vessels (Okayama M *et al.*, 1992)).

H<sub>1</sub> receptors are coupled to G<sub>q</sub> and, upon activation, stimulate phospholipase C to cleave membrane phospholipid into inositol-1,4,5-trisphosphate and 1,2-diacylglycerol, which promote an increase in  $[Ca^{2+}]_{intracellular}$  and activate PKC, respectively (Togias A, 2003). In vascular smooth muscle cells, these two pathways would stimulate the activation of the contractile machinery, thus causing vasoconstriction (Walsh MP, 1994). In endothelial cells, an increase in  $[Ca^{2+}]_{intracellular}$  has been implicated with the release of diffusible mediators from the endothelium which reduce smooth muscle cell  $[Ca^{2+}]_{intracellular}$  and thereby cause vasodilation (Chen GF *et al.*, 1990; Archer SL *et al.*, 1991; Busse R *et al.*, 2002). Histamine has been shown to cause a transient rise in  $[Ca^{2+}]_{intracellular}$  in cultured human nasal mucosal vascular endothelial cells (Ikeda H *et al.*, 1997).

Many of the studies investigating the endothelium-dependent H<sub>1</sub>-mediated vasodilation have shown it to be reduced by inhibitors of nitric oxide (NO) synthase (Fujiwara M *et al.*, 1992; Bockman CS *et al.*, 2002; Yousif MH *et al.*, 2002), suggesting that the H<sub>1</sub>-induced endothelium-derived relaxing factor (EDRF) was NO. However, nasal pretreatment with either L-NAME or N<sub>g</sub>-monomethyl-L-arginine (L-NMMA) did not inhibit histamine-induced nasal blockage in human subjects (Dear JW *et al.*, 1996a). As histamine-induced nasal blockage is reduced by H<sub>1</sub> antagonists, this suggests that NO is not a significant component of the H<sub>1</sub>-induced EDRF-mediated vasodilation. Ortiz *et al.* (1992) showed that only a combination of indomethacin (cyclooxygenase (COX) inhibitor) and an NO synthase inhibitor could reduce the histamine-induced EDRF-mediated vasodilation in human pulmonary arteries. Prostacyclin (PGI<sub>2</sub>), a product of endothelial cyclooxygenase, is a potent dilator of vascular smooth muscle (Busse R *et al.*, 1984; Klockenbusch W *et al.*, 1992) and has been found to be responsible for H<sub>1</sub>-mediated vasodilation in human umbilical arteries *in vitro* (Schellenberg RR *et al.*, 1986). The only study to investigate the effect of a COX inhibitor on histamine-induced nasal symptoms reported no reduction in nasal blockage (McLean JA *et al.*, 1983), although the same study reported a reduction in allergen-induced nasal blockage which counters

most reports in the literature which claim that inhibition of COX has no effect on allergen-induced symptoms (Brooks CD *et al.*, 1984; Walden SM *et al.*, 1988). A third candidate for contributing to the H<sub>1</sub>-induced vasodilation is endothelium-derived hyperpolarizing factor (EDHF). Now thought to be not just a single diffusible endothelial-derived mediator but an entire pathway, EDHF has been shown to contribute to vasodilation in many blood vessels (Yousif MH *et al.*, 2002; Busse R *et al.*, 2002), although its relevance tends to be inversely related to blood vessel size (Shimokawa H *et al.*, 1996). A rise in endothelial  $[Ca^{2+}]_{intracellular}$  activates  $Ca^{2+}$ -sensitive  $K^+$  channels, leading to hyperpolarization of the endothelial cell. This hyperpolarization spreads to the smooth muscle cell, possibly via myo-endothelial gap junctions, synthesis of epoxyeicosatrienoic acids (EETs) from cytochrome P450, activation of ion pumps in the smooth muscle cells, or a combination of any of the three (Busse R *et al.*, 2002). Whatever the precise mechanism is, EDHF results in vasodilation. Unfortunately, no studies have been published that have investigated EDHF-mediated vasodilation in nasal mucosa.

Endothelial H<sub>1</sub> receptors may mediate vasodilation in the human nasal mucosa by releasing NO, products of COX, EDHF, or a combination of any of the three.

H<sub>1</sub> receptors are also found on sensory neurones and it is these receptors which mediate the pruritus and sneezing caused after histamine nasal challenge (Mygind N, 1982). Activation of sensory neurones can lead to antidromic release of vasoactive neuropeptides. As mentioned in Chapter 1.7.1, substance P and CGRP are capable of causing nasal blockage in man (Chatelain C *et al.*, 1995). It is conceivable that some of the H<sub>1</sub>-induced nasal blockage is mediated indirectly via these neuropeptides. A study of vasodilation in guinea-pig nasal mucosa showed that histamine-induced vasodilation was inhibited by a CGRP receptor antagonist (Malis DD *et al.*, 2001). However, nasal pretreatment with phosphoramidon (reduces kinin and neuropeptide breakdown) has no effect on histamine-induced nasal blockage in non-atopic individuals at doses which augment bradykinin-induced nasal blockage (Turner PJ, 1999), and, in another study, histamine nasal challenge failed to cause the release of neuropeptides (Mosimann BL *et al.*, 1993), suggesting that neuropeptides do not contribute significantly to histamine-induced nasal blockage in man.

H<sub>1</sub> receptors on sympathetic nerve terminals have been demonstrated in guinea-pig nasal mucosa (Kubo N *et al.*, 1989b). Activation of these receptors leads to an increase in the release of NA, the vasoconstrictor. If this occurs in the human nasal mucosa, activation of these H<sub>1</sub> receptors would cause a decongestive effect. Clearly histamine, in man, causes nasal blockage (which is reduced by H<sub>1</sub> antagonists), not decongestion, thus if this H<sub>1</sub>-mediated mechanism does occur, it is obviously not as potent as the H<sub>1</sub>-mediated vasodilation. The significance, in man, of these putative sympathetic H<sub>1</sub> receptors is not known.

As mentioned earlier, H<sub>1</sub> antagonists, administered both topically and orally, reduce histamine-induced nasal blockage. However, the extent to which H<sub>1</sub> antagonists can reduce histamine-induced nasal blockage in man has not been previously investigated. Figures 3.12 and 3.13 (dose-response curves for the inhibition of 400µg histamine-induced nasal blockage by oral chlorpheniramine and cetirizine, respectively) suggested that a maximally effective dose of H<sub>1</sub> antagonist (in inhibiting histamine, 400µg) can be reached by increasing the antagonist dose to approximately twice the recommended clinical oral dose. There appeared to be no further reduction in the histamine-induced nasal blockage by increasing the dose of cetirizine above 20mg (clinical dose 10mg) or chlorpheniramine above 8mg (clinical dose 4mg). Figure 3.14 confirmed 20mg cetirizine as being the maximally effective dose of H<sub>1</sub> antagonist in reducing 400µg histamine-induced nasal blockage. Finally, Figure 3.15 showed that in the presence of a maximally effective dose of cetirizine, significant residual nasal blockage was evident. It is clear from these experiments that histamine causes nasal blockage by at least two separate mechanisms, one sensitive to cetirizine and one insensitive to cetirizine.

That 20mg cetirizine is the maximally effective dose in eliminating H<sub>1</sub>-mediated nasal blockage is further confirmed by published plasma levels of oral cetirizine (Urien S *et al.*, 1999). Assuming the Law of Mass Action applies and given the dissociation equilibrium constant of cetirizine at the H<sub>1</sub> receptor (~10nM), cetirizine at 20mg occupies >99% of H<sub>1</sub> receptors.

Histamine diphosphate, when dissolved in saline, causes a net increase in H<sup>+</sup> ions in an aqueous solution. Acidic solutions are known to cause irritation to mucosal surfaces (Worth AP *et al.*, 2001). Therefore, it was possible that the difference in pH

between the saline controls and 400µg histamine aerosols was responsible for the residual nasal blockage. However, nasal challenge with saline adjusted to the pH equivalent to that of a 4mg/ml histamine solution had no effect on nasal patency. The minor difference in pH between normal saline and histamine, 4mg/ml, is clearly not responsible for the cetirizine-insensitive nasal blockage caused by 400µg histamine.

### 3.5 Summary

- 1) Histamine, 40 to 400µg, induced a significant increase in nasal symptoms in normal individuals, observed as an increase in sneeze counts and symptom scores for rhinorrhea and congestion.
- 2) Histamine, 100 to 400µg, induced a significant decrease in the patency of the nasal airways of normal individuals, observed as an increase in nasal blockage.
- 3) Unilateral histamine, 400µg, induced a significant increase in nasal blockage in the ipsilateral nasal cavity, but had no effect on the contralateral nasal cavity.
- 4) Pretreatment with either topical chlorpheniramine, 1mg, oral chlorpheniramine, 8 to 16mg, or oral cetirizine, 20 to 30mg, significantly reduced the nasal blockage caused by histamine, 400µg.
- 5) Following pretreatment with maximally effective dose of cetirizine (20mg), histamine causes a significant nasal blockage.
- 6) The data indicate that histamine causes nasal blockage in normal individuals via H<sub>1</sub>-mediated and non-H<sub>1</sub>-mediated mechanisms.

# CHAPTER 4

## THE ROLE OF THE HISTAMINE H<sub>2</sub> RECEPTOR IN HISTAMINE-INDUCED NASAL BLOCKAGE

### 4.1 Introduction

Historically, H<sub>2</sub> receptors were thought to mediate the effects of histamine on cardiac muscle, uterine smooth muscle and gastric acid production. However, researchers discovered that H<sub>1</sub> antagonists were sometimes unable to abolish histamine's effect on the vasculature (Saxena PR, 1975; Powell JR *et al.*, 1976a; Dachman WD *et al.*, 1994). In Chapter 3, H<sub>1</sub> antagonists were shown to reduce, but not abolish, histamine-induced nasal blockage in non-atopic human subjects. H<sub>2</sub> receptor mRNA has been found in the nasal mucosa (Hirata N *et al.*, 1999a), and it is possible that the residual histamine-induced nasal blockage, when in the presence of an H<sub>1</sub> antagonist, is mediated by H<sub>2</sub> receptors.

The two H<sub>2</sub> antagonists used in this chapter, ranitidine and famotidine, are both potent and highly selective for H<sub>2</sub> receptors (Bertaccini G *et al.*, 1986; Mills JG *et al.*, 1989; van der Goot H *et al.*, 2000).

In this chapter, the contribution of H<sub>2</sub> receptors to the nasal blockage caused by histamine after it has been sprayed into the nasal cavities of normal, non-atopic, human subjects, has been investigated.



## 4.2 Experimental protocol

In the following double-blind experiments, healthy non-atopic human volunteers were used as subjects.

### 4.2.1 The effect of topical histamine H<sub>2</sub> agonist, dimaprit, on nasal patency

The aim of this experiment was to investigate the effect of an H<sub>2</sub> agonist on nasal patency, as measured by acoustic rhinometry. Subjects received, by 100µl aerosol into each nostril, either saline or dimaprit, 50 or 200µg. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The doses and time-course used were based upon comparisons with histamine and the published potency of the H<sub>2</sub> agonist, dimaprit (Parsons ME *et al.*, 1977). The protocol is summarized in Figure 4.1.

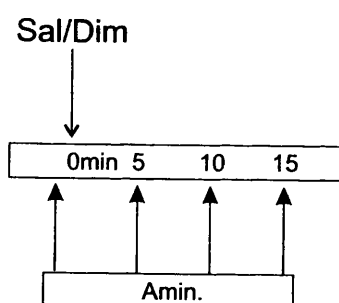


Figure 4.1: Protocol for the effect of dimaprit on nasal patency. Sal = saline, Dim = dimaprit.

### 4.2.2 The effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on dimaprit-induced nasal blockage

The aim of this experiment was to determine if dimaprit, the H<sub>2</sub> agonist, caused nasal blockage via H<sub>2</sub> receptors. Subjects received oral ranitidine, 150mg, or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or dimaprit, 200µg. Each subject received the following 3 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; dimaprit, 200µg
- iii) Ranitidine, 150mg; dimaprit, 200µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The doses and time-course (pretreatment time and interval between separate treatments) of this study was based on the published pharmacokinetics of ranitidine (McNeil JJ *et al.*, 1981; van Hecken AM *et al.*, 1982). The protocol is summarized in Figure 4.2.

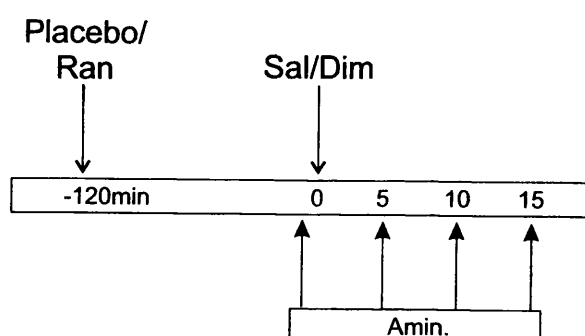


Figure 4.2: Protocol for investigating the effect of ranitidine on dimaprit-induced nasal blockage. Ran = ranitidine, Sal = saline, Dim = dimaprit.

#### 4.2.3 The effect of oral histamine H<sub>2</sub> antagonists on histamine-induced nasal blockage

The aim of this experiment was to assess the degree to which histamine-induced nasal blockage was mediated via H<sub>2</sub> receptors. Subjects received oral ranitidine, 32.5, 75 or 150mg, or oral famotidine, 10 or 30mg, or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 7 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Ranitidine, 32.5mg; histamine, 400µg
- iv) Ranitidine, 75mg; histamine, 400µg
- v) Ranitidine, 150mg; histamine, 400µg

- vi) Famotidine, 10mg; histamine, 400µg
- vii) Famotidine, 30mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (A<sub>min.</sub>) of the nasal airways was measured using acoustic rhinometry. 12 subjects were included in this study. The protocol is summarized in Figure 4.3.

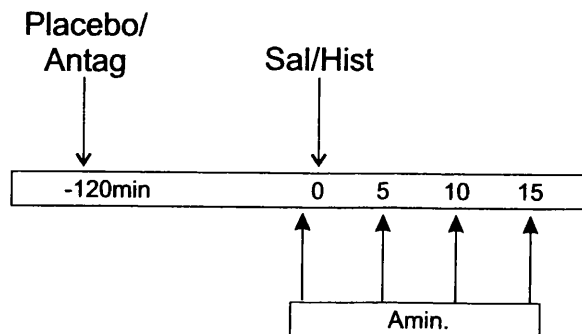


Figure 4.3: Protocol for investigating the effect of oral H<sub>2</sub> antagonists on histamine-induced nasal blockage. Antag = H<sub>2</sub> antagonist, Sal = saline, Hist = histamine.

#### 4.2.4 The effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>1</sub> antagonist, cetirizine

The aim of this experiment was to assess whether or not an H<sub>2</sub> antagonist would augment the reduction of histamine-induced nasal blockage by H<sub>1</sub> antagonists. Subjects received oral cetirizine, 5, 20 or 30mg, or oral placebo, with or without oral ranitidine, 150mg. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 9 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Ranitidine, 150mg; histamine, 400µg
- iv) Cetirizine, 5mg; histamine, 400µg
- v) Cetirizine, 20mg; histamine, 400µg
- vi) Cetirizine, 30mg; histamine, 400µg
- vii) Cetirizine, 5mg, and ranitidine, 150mg; histamine, 400µg
- viii) Cetirizine, 20mg, and ranitidine, 150mg; histamine, 400µg

ix) Cetirizine, 30mg, and ranitidine, 150mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 7 subjects were included in this study. The protocol is summarized in Figure 4.4.

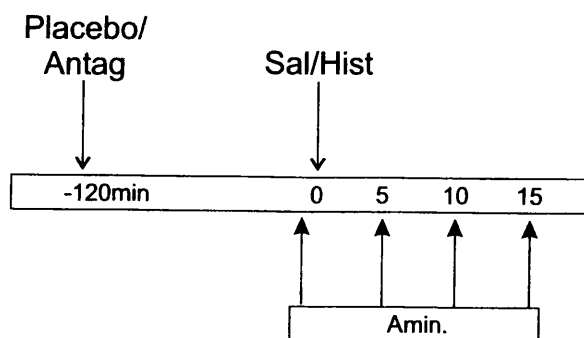


Figure 4.4: Protocol for investigating the effect of oral H<sub>2</sub> antagonist on the reduction of histamine-induced nasal blockage by oral H<sub>1</sub> antagonist. Antag = antagonist, Sal = saline, Hist = histamine.

#### 4.2.5 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, and oral histamine H<sub>2</sub> antagonist, ranitidine, alone or in combination, on histamine-induced nasal blockage

The aim of this experiment was to determine whether a combination of H<sub>1</sub> and H<sub>2</sub> antagonists would be more effective in reducing histamine-induced nasal blockage than an H<sub>1</sub> antagonist alone; and to determine if any residual nasal blockage was present after pretreatment with the antagonist combination. Subjects received, alone or in combination, oral cetirizine, 20mg, oral ranitidine, 75mg, or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 5 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Ranitidine, 75mg; histamine, 400µg
- iv) Cetirizine, 20mg; histamine, 400µg
- v) Ranitidine, 75mg and cetirizine, 20mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 16 subjects were included in this study. The protocol is summarized in Figure 4.5.

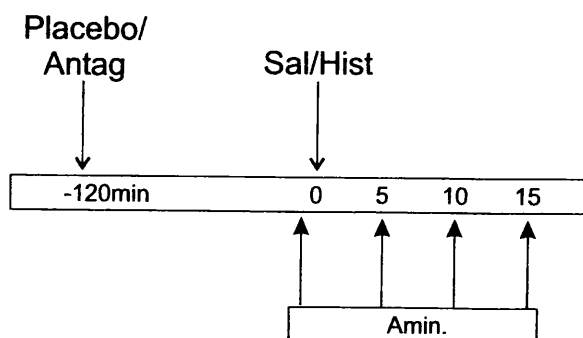


Figure 4.5: Protocol for investigating the effect of oral H<sub>1</sub> and H<sub>2</sub> antagonists, alone or in combination, on histamine-induced nasal blockage. Antag = antagonist, Sal = saline, Hist = histamine.

#### 4.2.6 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>2</sub> antagonist, ranitidine

The aim of this experiment was to assess whether or not an H<sub>1</sub> antagonist would augment the reduction of histamine-induced nasal blockage by H<sub>2</sub> antagonists. Subjects received oral ranitidine, 75 or 300mg, or oral placebo, with or without oral cetirizine, 20mg. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 7 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Cetirizine, 20mg; histamine, 400µg
- iv) Ranitidine, 75mg; histamine, 400µg
- v) Ranitidine, 300mg; histamine, 400µg
- vi) Ranitidine, 75mg and cetirizine, 20mg; histamine, 400µg
- vii) Ranitidine, 300mg and cetirizine, 20mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic

rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 4.6.

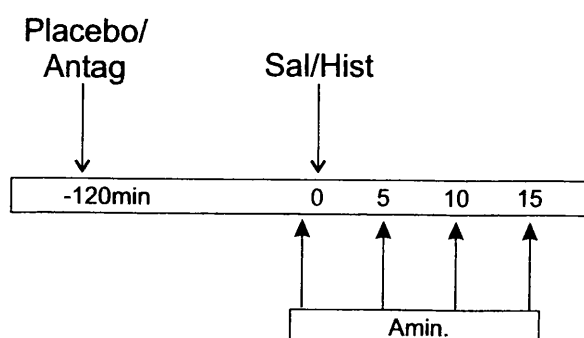


Figure 4.6: Protocol for investigating the effect of oral H<sub>1</sub> antagonist on the reduction of histamine-induced nasal blockage by oral H<sub>2</sub> antagonist. Antag = antagonist, Sal = saline, Hist = histamine.

#### 4.2.7 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, on histamine-induced nasal blockage in the presence of oral histamine H<sub>2</sub> antagonist, ranitidine

The aim of this experiment was to investigate whether increasing the ranitidine dose above 75mg significantly increased the reduction of histamine-induced nasal blockage; and to determine the degree of residual histamine-induced nasal blockage in the presence of maximal doses of H<sub>1</sub> and H<sub>2</sub> antagonists. Subjects received oral ranitidine, 75 or 150mg, with or without oral cetirizine, 20mg. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 6 treatments in a random order, with at least 48 hours between each treatment:

- i) Ranitidine, 75mg; saline
- ii) Ranitidine, 150mg; saline
- iii) Ranitidine, 75mg; histamine, 400µg
- iv) Ranitidine, 150mg; histamine, 400µg
- v) Ranitidine, 75mg and cetirizine, 20mg; histamine, 400µg
- vi) Ranitidine, 150mg and cetirizine, 20mg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic

rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 4.7.

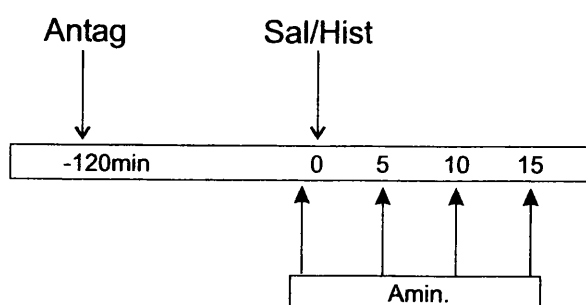


Figure 4.7: Protocol for investigating the effect of oral H<sub>1</sub> antagonist on histamine-induced nasal blockage in the presence of oral H<sub>2</sub> antagonist. Antag = antagonist, Sal = saline, Hist = histamine.

#### 4.2.8 Meta-analysis of the effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on histamine-induced nasal blockage

The aim of this meta-analysis was to group together all the experiments investigating the effect of ranitidine (H<sub>2</sub> antagonist) and determine its effectiveness in reducing histamine-induced nasal blockage. All studies which investigated the effect of oral ranitidine on histamine-induced nasal blockage were included. Data representing the effect of ranitidine in combination with cetirizine was not included. Thus only the following data points from each study were included (if available):

- i) Placebo; saline
- ii) Placebo; histamine, 400µg
- iii) Ranitidine, 32.5mg; histamine, 400µg
- iv) Ranitidine, 75mg; histamine, 400µg
- v) Ranitidine, 150mg; histamine, 400µg
- vi) Ranitidine, 300mg; histamine, 400µg

The meta-analysis comprised of 7 studies (A to G): A (n=8), B (n=7), C (n=8), D (n=8), E (n=16), F (n=10) and G (n=12). The studies did not always involve the same subjects, although some subjects took part in more than one study.

Each study followed an identical protocol: subjects received oral ranitidine or oral placebo. 2 hours later, subjects received, by 100 $\mu$ l aerosol into each nostril, saline or histamine, 400 $\mu$ g. Each subject received the treatments in a random order, with at least 48 hours between each treatment. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. The protocol is summarized in Figure 4.8.

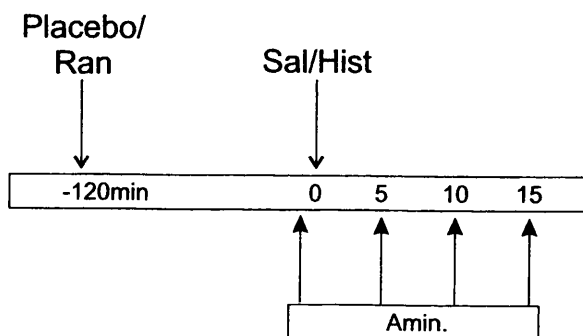


Figure 4.8: Protocol for investigating the effect of oral  $H_2$  antagonist, ranitidine, on histamine-induced nasal blockage. Ran = ranitidine, Sal = saline, Hist = histamine.

#### 4.2.9 Data analysis

Nasal patency was measured using acoustic rhinometry, as described in Chapter 2. Amin. was recorded immediately prior to and 5, 10 and 15 minutes after nasal challenge. Amin. values were normalised to the Amin. value recorded prior to nasal challenge. For each challenge, a normalised Amin. versus time curve was plotted, and the area under the curve (AUC) calculated. The rhinometry data are presented either as mean values, together with s.e.mean; or as individual data. The appropriate non-parametric statistical test is given with each data set.  $p < 0.05$  is taken as significant.



## 4.3 Results

### 4.3.1 The effect of topical histamine H<sub>2</sub> agonist, dimaprit, on nasal patency

Application, by aerosol into each nostril, of dimaprit, 200µg, caused a significant reduction in AUC (area under the normalised Amin. versus time curve) as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge, as shown in Figure 4.9.

### 4.3.2 The effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on dimaprit-induced nasal blockage

Application, by aerosol into each nostril, of dimaprit, 200µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral ranitidine, 150mg, 2 hours prior to nasal challenge, abolished the reduction in AUC by dimaprit ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 4.10).

### 4.3.3 The effect of oral histamine H<sub>2</sub> antagonists on histamine-induced nasal blockage

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge.

Pretreatment with oral ranitidine, 32.5, 75 and 150mg, 2 hours prior to nasal challenge, had no effect on the reduction in AUC caused by histamine ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 4.11).

Pretreatment with oral famotidine, 10 and 30mg, 2 hours prior to nasal challenge, had no effect on the reduction in AUC caused by histamine ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 4.12).

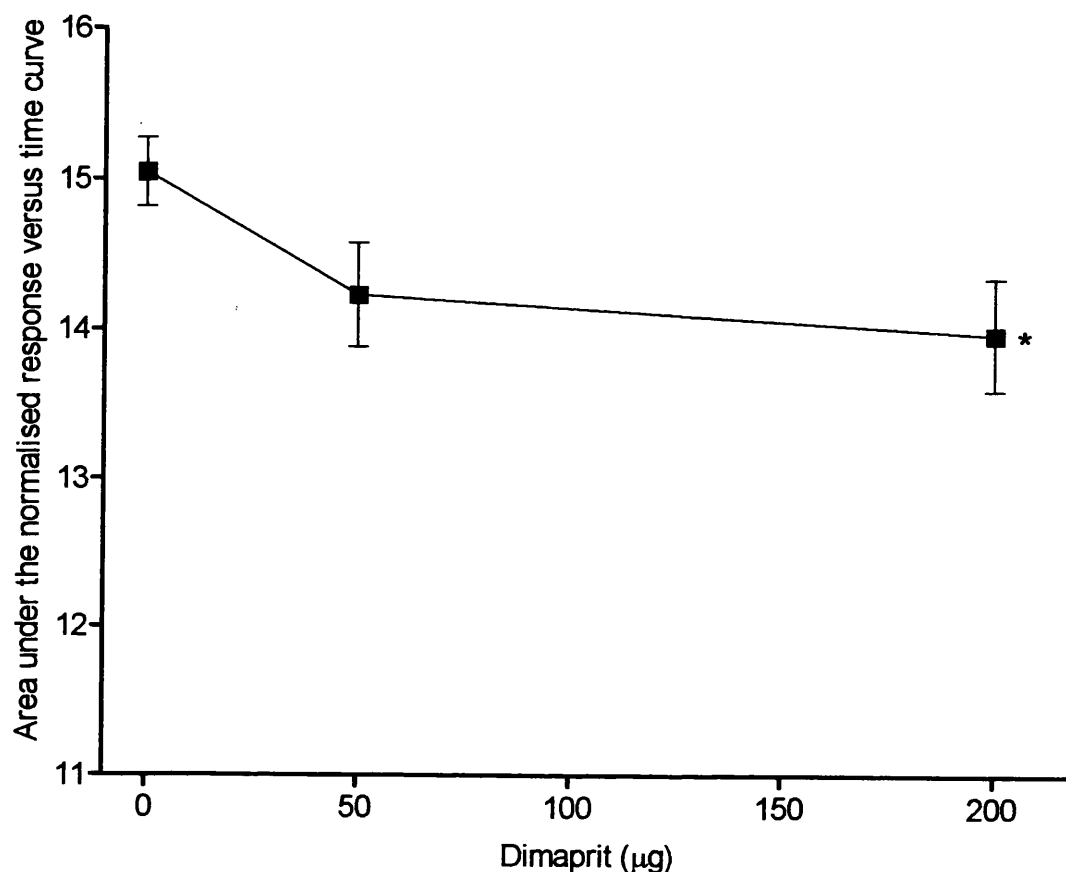


Figure 4.9: Dose-response curve for the action of dimaprit on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of dimaprit as an aerosol, at the dose shown, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.726cm<sup>2</sup>, s.e.mean of 0.056cm<sup>2</sup>). Data are means from 8 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

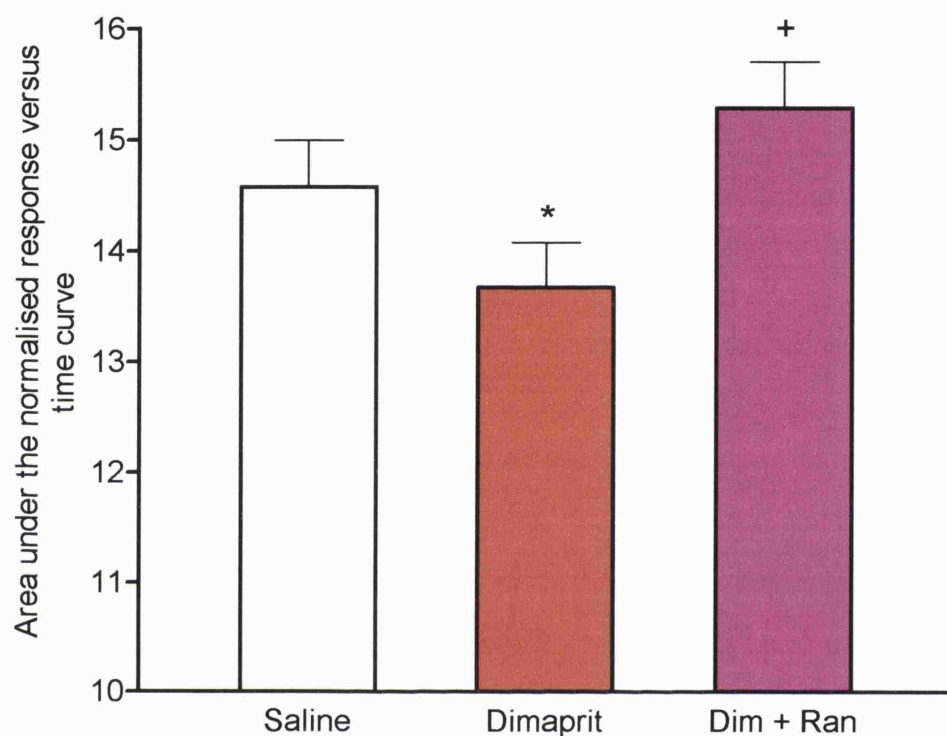


Figure 4.10: The effect of ranitidine [Ran], 150mg, on the nasal blockage caused by dimaprit [Dim], 200µg, administered as an aerosol into each nostril. Ranitidine, 150mg, was given orally 2 hours prior to dimaprit challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.675cm<sup>2</sup>, s.e.mean of 0.050cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of dimaprit. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to dimaprit without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

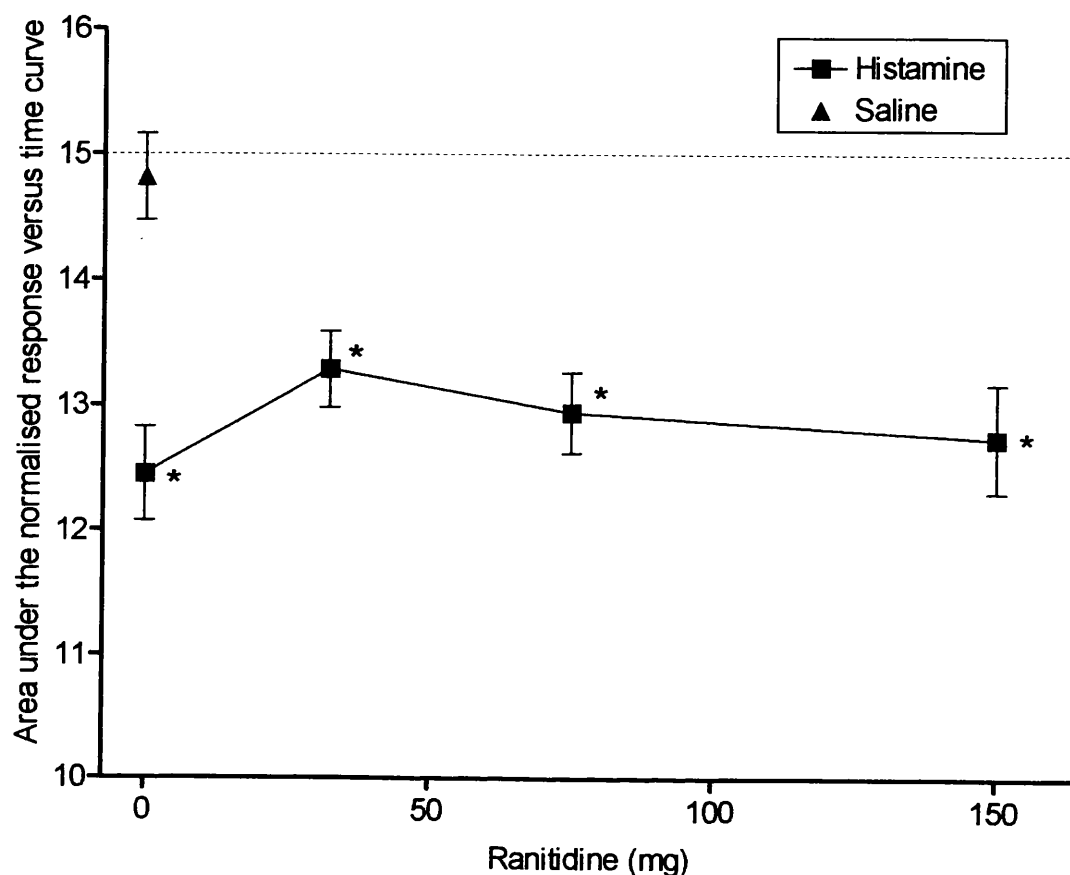


Figure 4.11: Dose-response curve for the inhibition by orally administered ranitidine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.799cm<sup>2</sup>, s.e.mean of 0.030cm<sup>2</sup>). The data are the means from 12 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by oral H<sub>2</sub> antagonists (Figure 4.17) (Havas TE *et al.*, 1986). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

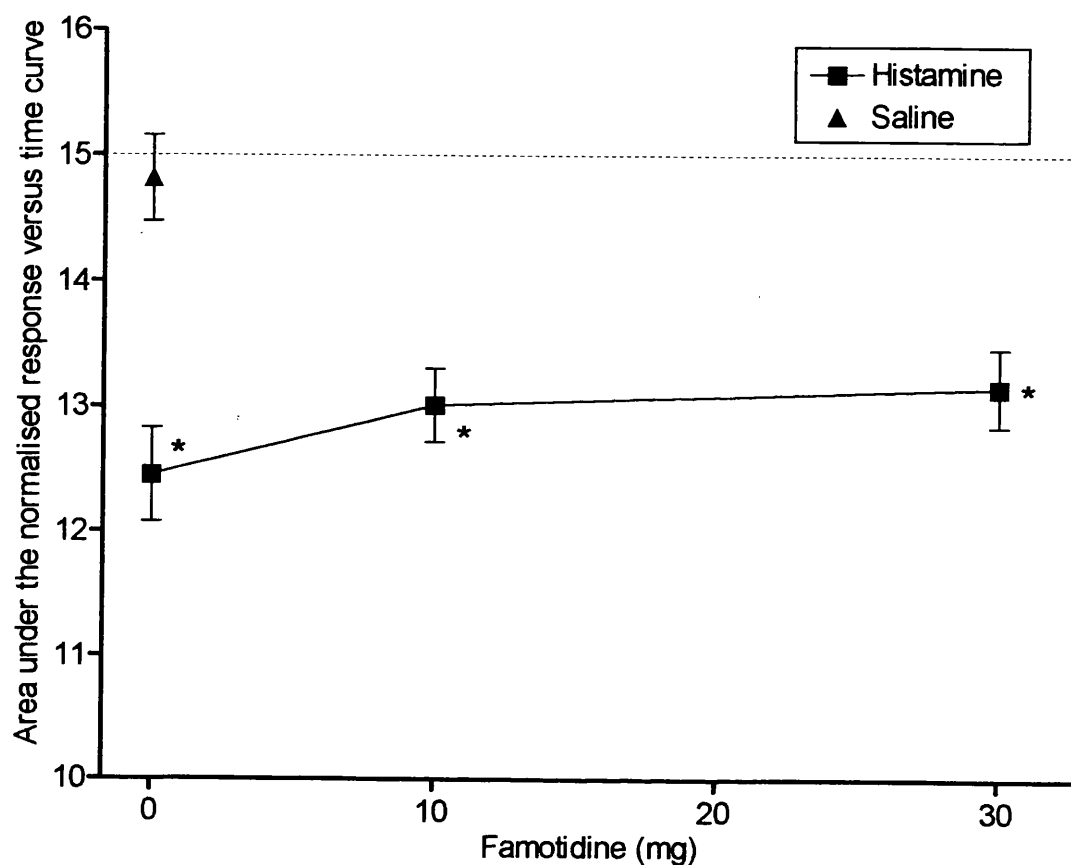


Figure 4.12: Dose-response curve for the inhibition by orally administered famotidine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.799cm<sup>2</sup>, s.e.mean of 0.030cm<sup>2</sup>). The data are the means from 12 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by oral H<sub>2</sub> antagonists (Figure 4.17) (Havas TE *et al.*, 1986). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

#### **4.3.4 The effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>1</sub> antagonist, cetirizine**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral ranitidine, 150mg, alone, 2 hours prior to nasal challenge, had no effect on the reduction in AUC caused by histamine ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 4.13).

Pretreatment with oral cetirizine, 20 and 30mg, alone, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test).

Pretreatment with oral cetirizine, 5mg, and oral ranitidine, 150mg, both given 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine with ranitidine pretreatment alone ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test).

Pretreatment with oral cetirizine, 20mg, and oral ranitidine, 150mg, both given 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine ( $p < 0.05$ , Wilcoxon sign-rank test) to the extent that no significant residual nasal blockage remained.

Pretreatment with oral cetirizine, 30mg, and oral ranitidine, 150mg, both given 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine, and as compared to cetirizine pretreatment alone ( $p < 0.05$ , Wilcoxon sign-rank test). No significant residual nasal blockage caused by histamine remained after pretreatment with the combination of cetirizine, 30mg and ranitidine, 150mg.

Both sets of data, histamine alone and histamine in the presence of ranitidine, were fitted by GraphPad Prism 3.0, using nonlinear regression, equation:

$$Y = \frac{B_{\max} \cdot X}{K_d + X} + 12.1$$

No weighting of the data was used. Convergence was reached when two consecutive iterations changed the sum of squares by less than 0.01%.

The response following histamine nasal challenge for each cetirizine dose (5, 20 and 30mg) (Table 4.1) was combined into a single value for each subject. This value was reached by first plotting AUC (area under the normalised Amin. versus time curve) against cetirizine dose, and then calculating the integral of AUC and cetirizine dose ( $\int_{30}^5 \text{AUC} \delta \text{cetirizine dose}$ ) for each subject (see insert, Figure 4.14). This value then represented the total effect of histamine, with pretreatment of cetirizine, 5mg through to 30mg, on the normalised Amin. over a 15 minute period after nasal challenge. The units of this value are unit.min.mg. This calculation was repeated with the histamine nasal challenge following ranitidine pretreatment data for each subject (Table 4.1). The total effect of histamine, with and without pretreatment of ranitidine, in the presence of cetirizine, 5mg through to 30mg, on the normalised Amin. over a 15 minute period after nasal challenge is shown in Figure 4.14.

Pretreatment with oral ranitidine, 150mg, 2 hours prior to nasal challenge, significantly increased the area under the AUC versus cetirizine dose curve as compared to without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to a significant further reduction in histamine-induced nasal blockage by a combination of cetirizine and ranitidine pretreatment as compared to the reduction caused by cetirizine pretreatment alone.

#### **4.3.5 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, and oral histamine H<sub>2</sub> antagonist, ranitidine, alone or in combination, on histamine-induced nasal blockage**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test),

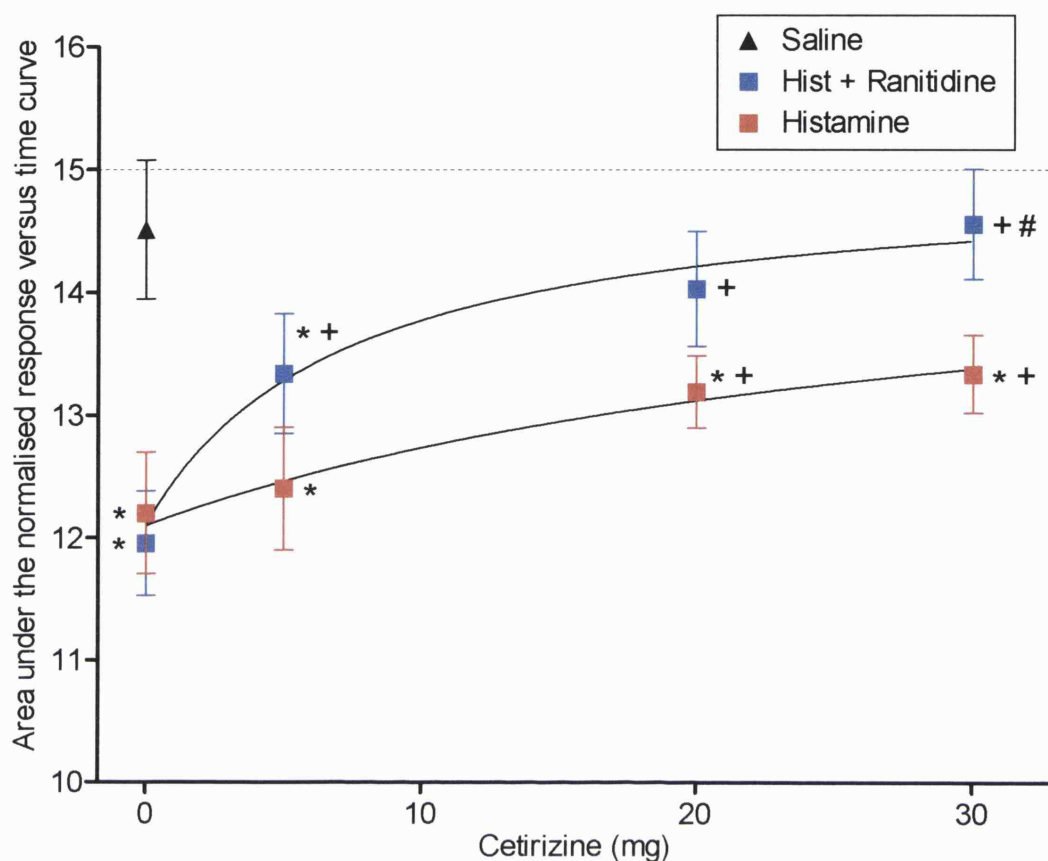


Figure 4.13: Dose-response curve for the inhibition by orally administered cetirizine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine [Hist], 400µg, administered as an aerosol into each nostril. ■ - histamine alone; ■ - histamine in the presence of ranitidine, 150mg, given orally 2 hours prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.576cm<sup>2</sup>, s.e.mean of 0.013cm<sup>2</sup>). The data are the means from 7 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. Curves fitted using nonlinear regression. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by oral H<sub>1</sub> and H<sub>2</sub> antagonists (Figures 3.11 and 4.17) (Havas TE *et al.*, 1986; Austin CE *et al.*, 1996). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage with cetirizine pretreatment as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in nasal blockage with ranitidine pretreatment as compared to histamine without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).



	Histamine			Histamine with ranitidine		
	Cetirizine (mg)			Cetirizine (mg)		
	5	20	30	5	20	30
Subject 1	13.17	14.32	12.75	13.98	14.11	13.91
Subject 2	11.5	13.96	12.54	10.35	12.65	12.08
Subject 3	12.37	12.68	13.94	14.17	16.47	15.08
Subject 4	13.56	12.95	12.51	13.39	14.66	15.59
Subject 5	10.07	12.91	15.09	13.22	13.18	16.34
Subject 6	11.87	11.9	13.82	14.18	12.85	14.63
Subject 7	14.3	13.63	12.8	14.1	14.3	14.41

Table 4.1: Dose-response curve for the inhibition by orally administered cetirizine, alone or in the presence of ranitidine, of nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. Both cetirizine, 5, 20 and 30mg, and ranitidine, 150mg, were given orally 2 hours prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.576cm<sup>2</sup>, s.e.mean of 0.013cm<sup>2</sup>). The data values are the responses from 7 individuals and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine.

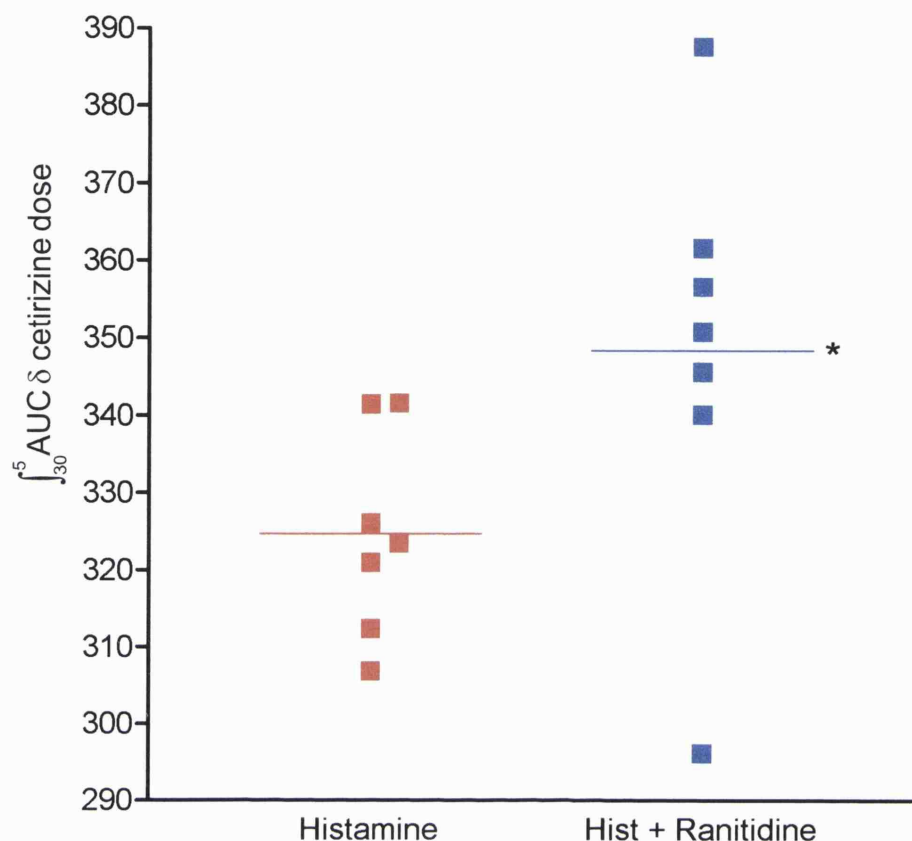


Figure 4.14: Graph shows the effective reduction by ranitidine, 150mg, of the nasal blockage caused by histamine [Hist], 400µg, administered as an aerosol into each nostril, in the presence of cetirizine (5, 20 or 30mg). Both cetirizine and ranitidine were given orally 2 hours prior to histamine challenge. The response [AUC] of each individual to histamine in the presence of 3 doses of cetirizine (5, 20 or 30mg) (Table 4.1), was plotted against

cetirizine dose (see insert for example using Subject 6 data) and the integral

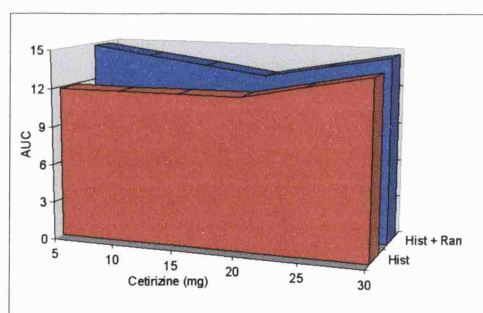
(  $\int_{30}^5 \text{AUC } \delta \text{ cetirizine dose}$  ) was calculated

(306.9 unit.min.mg for Subject 6). Thus the

responses for histamine after pretreatment with the three doses of cetirizine (■) were pooled into one value for each individual that

corresponds to the total effect of histamine in the presence of cetirizine, 5mg through to 30mg. In addition, the response [AUC] of each individual to histamine in the presence of 3 doses of cetirizine (5, 20 or 30mg) in combination with ranitidine, 150mg, (Table 4.1), was plotted against cetirizine dose (see insert for example using Subject 6 data) and the integral

(  $\int_{30}^5 \text{AUC } \delta \text{ cetirizine dose}$  ) was calculated (340.1 unit.min.mg for Subject 6). Thus the



responses for histamine after pretreatment with the three doses of cetirizine in the presence of ranitidine (■) were also pooled into one value for each individual that corresponds to the total effect of histamine in the presence of ranitidine and cetirizine, 5mg through to 30mg. The data in Figure 4.14 are the calculated responses from 7 individuals and represent the integral of AUC (area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine) and cetirizine dose:  $\int_{30}^5 \text{AUC} \delta \text{cetirizine dose}$ . Horizontal bars represent the mean. \* Significant reduction in nasal blockage as compared to histamine without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with either oral cetirizine, 20mg, or oral ranitidine, 75mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test). Pretreatment with a combination of both oral cetirizine, 20mg, and oral ranitidine, 75mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatment, and as compared to histamine with pretreatment of either antagonist alone ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 4.15).

None of the pretreatments fully abolished the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test).

#### **4.3.6 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>2</sub> antagonist, ranitidine**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with either oral cetirizine, 20mg, or oral ranitidine, 75 or 300mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test). Pretreatment with a combination of oral ranitidine, 75 or 300mg, and cetirizine, 20mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatment, and as compared to histamine with cetirizine pretreatment alone ( $p < 0.05$ , Wilcoxon sign-rank test). However, combined ranitidine (either 75 or 300mg) and cetirizine pretreatment had no effect on AUC caused by histamine as compared to histamine with ranitidine pretreatment alone ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 4.16).

None of the pretreatment combinations fully abolished the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test).

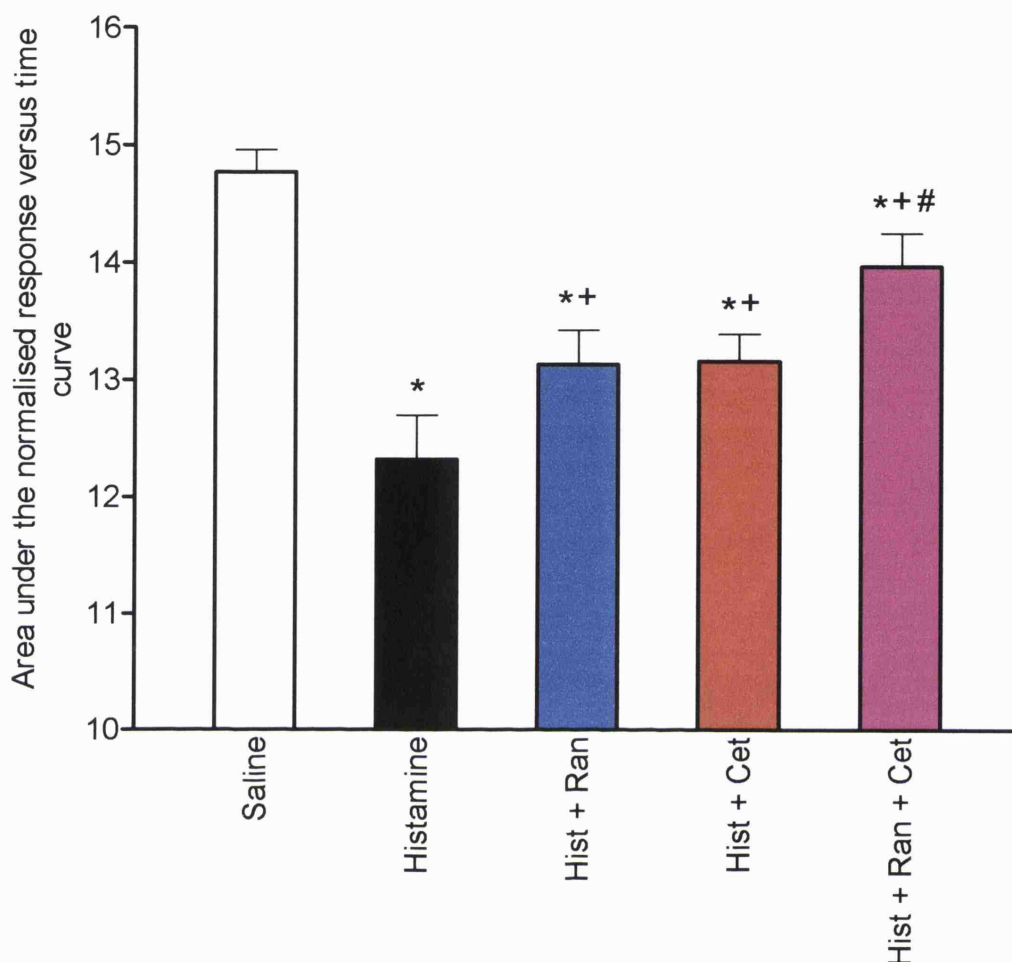


Figure 4.15: The effect of ranitidine [Ran], 75mg and cetirizine [Cet], 20mg alone and in combination, on the nasal blockage caused by histamine [Hist], 400µg, given as an aerosol into each nostril. Both drugs were given orally 2 hours prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.729cm<sup>2</sup>, s.e.mean of 0.027cm<sup>2</sup>). The data are the means from 16 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without ranitidine or cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in nasal blockage as compared to histamine with cetirizine alone pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

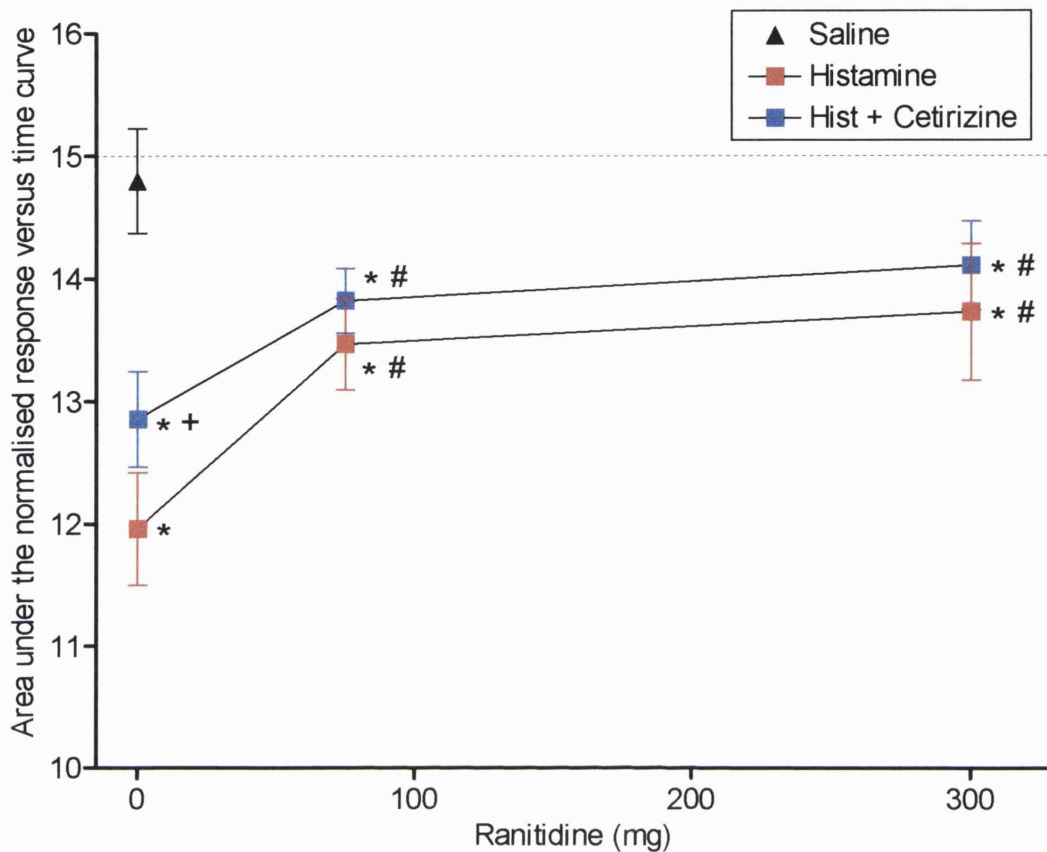


Figure 4.16: Dose-response curve for the inhibition by orally administered ranitidine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine [Hist], 400 $\mu$ g, administered as an aerosol into each nostril. ■ - histamine alone; ■ - histamine in the presence of cetirizine, 20mg, given orally 2 hours prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.619cm<sup>2</sup>, s.e.mean of 0.025cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The dotted line represents the effect of saline nasal challenge on the area under the normalised Amin. versus time curve measured over a 15 minute period, based on the assumption that the nasal response to saline is unaffected by oral H<sub>1</sub> and H<sub>2</sub> antagonists (Figures 3.11 and 4.17) (Havas TE *et al.*, 1986; Austin CE *et al.*, 1996). \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage with cetirizine pretreatment as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in nasal blockage with ranitidine pretreatment as compared to histamine without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

#### **4.3.7 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, on histamine-induced nasal blockage in the presence of oral histamine H<sub>2</sub> antagonist, ranitidine**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline when pretreated with 75mg or 150mg ranitidine, given 2 hours prior to nasal challenge ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 4.17).

Pretreatment with a combination of cetirizine, 20mg and ranitidine, 150mg, 2 hours prior to nasal challenge, caused a significant inhibition of the reduction in AUC by histamine as compared to histamine with ranitidine pretreatment, 150mg, alone ( $p < 0.05$ , Wilcoxon sign-rank test). However, pretreatment with a combination of cetirizine, 20mg and ranitidine, 75mg, 2 hours prior to nasal challenge, had no effect on the AUC caused by histamine as compared to histamine with ranitidine pretreatment, 75mg, alone ( $p > 0.05$ , Wilcoxon sign-rank test).

Increasing the ranitidine pretreatment dose from 75mg to 150mg had no effect on the AUC caused by saline or histamine (with or without cetirizine pretreatment) ( $p > 0.05$ , Wilcoxon sign-rank test).

None of the antagonist pretreatments fully abolished the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test).

#### **4.3.8 Meta-analysis of the effect of oral histamine H<sub>2</sub> antagonist, ranitidine, on histamine-induced nasal blockage**

The results in Chapter 4 showed that the effect of ranitidine alone on the reduction in AUC caused by histamine, 400µg, was equivocal. The purpose of the meta-analysis was to determine if any significant effect of ranitidine was exposed after all the data were pooled together.

The meta-analysis comprised of data taken from 7 separate studies (A to G). Only data representing the response of saline, histamine, and histamine pretreated with ranitidine was included. The data which represented the effect of histamine

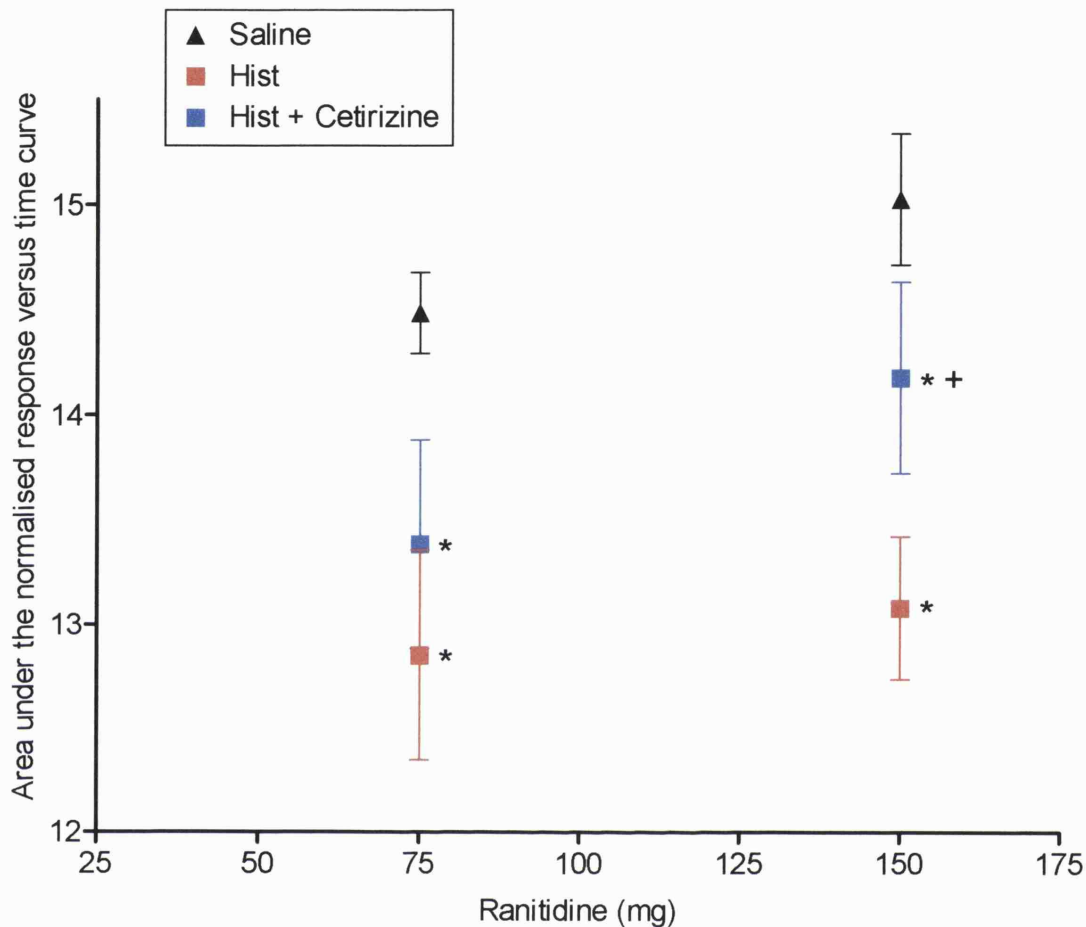


Figure 4.17: Dose-response curve for the inhibition by orally administered ranitidine, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine [Hist], 400µg, administered as an aerosol into each nostril. ■ - histamine alone; ■ - histamine in the presence of cetirizine, 20mg, given orally 2 hours prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.684cm<sup>2</sup>, s.e.mean of 0.031cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage with cetirizine pretreatment as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).



pretreated with other antagonists (e.g. H<sub>2</sub> antagonist, famotidine or H<sub>1</sub> antagonist, cetirizine) were excluded.

Figure 4.18 shows the effect in 7 separate studies (A to G) of saline, histamine, 400µg and histamine, 400µg, pretreated 2 hours prior to nasal challenge with oral ranitidine (either 32.5, 75, 150 or 300mg), on the AUC. In all studies, application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test); with the exception of experiment F, which did not include a histamine challenge without antagonist pretreatment. In addition, none of the antagonist pretreatments fully abolished the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test).

The studies had different n numbers and did not always involve the same subjects, although some subjects took part in more than one study. In addition, none of the studies included every dose of ranitidine. Therefore statistical analysis of the data could not be correctly performed using a repeated measures, non-parametric equivalent of the parametric two-way analysis of variance (ANOVA), e.g. Friedman's test. Instead, the data from all the experiments for each nasal challenge were pooled into the following groups:

- i) Placebo; saline (n=69)
- ii) Placebo; histamine, 400µg (n=60)
- iii) Ranitidine, 32.5mg; histamine, 400µg (n=20)
- iv) Ranitidine, 75mg; histamine, 400µg (n=54)
- v) Ranitidine, 150mg; histamine, 400µg (n=37)
- vi) Ranitidine, 300mg; histamine, 400µg (n=8)

For this to be justified, the data was analysed for any significant correlation between subject and AUC using the non-parametric Spearman's rank test (both with zero order partials and partial correlation, controlling for ranitidine dose). No significant correlation between subject and AUC was found with either test ( $p > 0.05$  Spearman's rank test, zero order partials) ( $p > 0.05$  Spearman's rank test, partial correlation controlling for dose), indicating that the result (AUC) of any given test (e.g. histamine pretreated with 75mg ranitidine) was not significantly dependent on

the individual subject (Figure 4.19). This allows the data to be pooled into the 6 groups shown above, as shown in Figure 4.20.

The combined dose response relationship for the inhibition, by oral ranitidine, given 2 hours prior to nasal challenge, of the reduction in AUC by histamine, 400µg, is shown in Figure 4.20. Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test). Pretreatment with oral ranitidine alone significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.01$ , Kruskal-Wallis test). Post-hoc Dunn's multiple comparisons test showed that there were no significant differences between the ranitidine groups, with the exception of a significant difference between ranitidine, 150mg, and ranitidine, 300mg ( $p < 0.05$ , Dunn's multiple comparisons test).

None of the antagonist pretreatments fully abolished the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test).

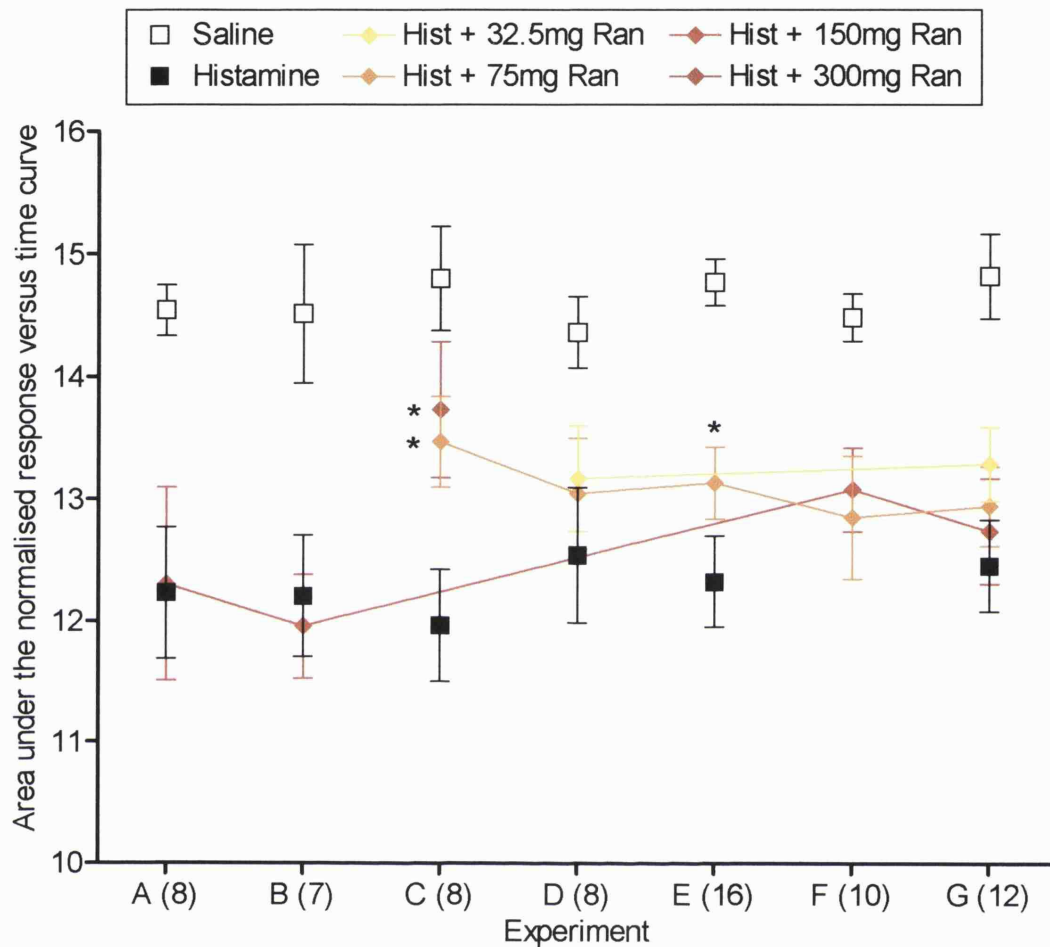


Figure 4.18: Overview of the inhibition by orally administered ranitidine [Ran], given 2 hours prior to histamine challenge, of nasal blockage caused by histamine, 400µg, administered as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.687cm<sup>2</sup>, s.e.mean of 0.016cm<sup>2</sup>). The number of subjects contributing to each data point is shown in parentheses. The data represent the mean area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant reduction in nasal blockage as compared to histamine without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

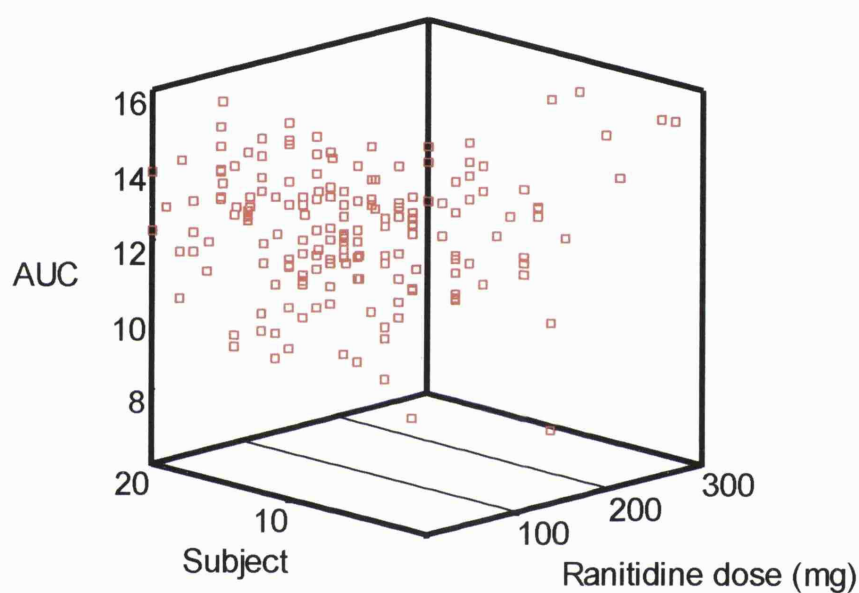


Figure 4.19: Three-Dimensional scatterplot showing the correlation between subject and the nasal blockage caused by histamine, 400µg, administered as an aerosol into each nostril, in the presence of ranitidine (0, 32.5, 75, 150 and 300mg). Ranitidine was given orally 2 hours prior to histamine challenge. Data points represent the area under the normalised response versus time curve measured over a 15 minute period following the administration of histamine [AUC] (n = 248).

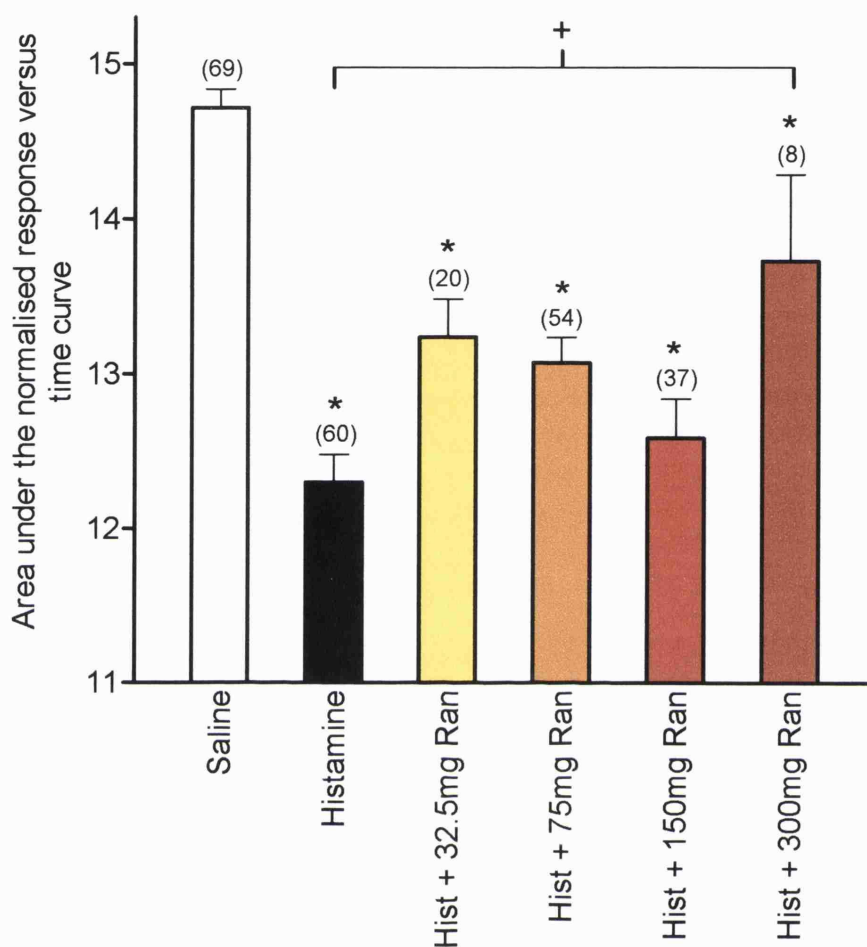


Figure 4.20: Combined dose-response curve for the inhibition by orally administered ranitidine [Ran], at the doses shown, given 2 hours prior to challenge with histamine, of nasal blockage caused by histamine [Hist], 400 $\mu$ g, administered as an aerosol into each nostril. Data has been combined from all the studies investigating the effect of orally administered ranitidine on the nasal blockage caused by histamine, 400 $\mu$ g, administered as an aerosol into each nostril. The data represent the overall mean area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. The number of subjects contributing to each data point is shown in parentheses. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in histamine-induced nasal blockage by ranitidine ( $p < 0.01$ , Kruskal-Wallis test). Post-hoc Dunn's multiple comparisons test was performed, examining the inter-group differences between doses of ranitidine. 5 of the 6 comparisons were non-significant, with the exception of Hist + 150mg Ran versus Hist + 300mg Ran ( $p < 0.05$ ).

#### 4.4 Discussion

Dimaprit, following bilateral nasal administration as an aerosol, 200µg, caused a significant reduction in the area under the normalised Amin. versus time curve measured over a period of 15 minutes (AUC). This reduction in AUC represents an objectively measured reduction in nasal patency or, alternatively, increased nasal blockage. That dimaprit, a selective H<sub>2</sub> agonist, caused nasal blockage is in agreement with a report that impromidine, the H<sub>2</sub> partial agonist, also caused nasal blockage when nasally administered to normal, non-atopic, individuals (Shelton D *et al.*, 1994). Dimaprit has been shown to inhibit neuronal nitric oxide synthase (NOS) (Paquay JB *et al.*, 1999), and it is possible that this mechanism is responsible for the nasal blockage. However, previous studies have shown that the non-selective NOS inhibitor L-NAME had no effect on resting nasal patency and actually *reduced* bradykinin-induced nasal blockage (Dear JW *et al.*, 1996a). Dimaprit-induced nasal blockage was abolished by pretreatment with ranitidine, the highly selective H<sub>2</sub> antagonist, indicating that the nasal blockage was, in fact, mediated by H<sub>2</sub> receptors alone.

Oral ranitidine had no effect on saline nasal challenge, indicating that H<sub>2</sub> antagonists have no effect on resting nasal patency, which is in agreement with the available literature (Havas TE *et al.*, 1986).

H<sub>2</sub> receptors are usually G<sub>s</sub>-coupled. H<sub>2</sub> mediated vasodilation has been reported in guinea-pig skin blood vessels (Owen DA *et al.*, 1980), human temporal arteries (Ottosson A *et al.*, 1989), cat pial arteries (Gross PM *et al.*, 1981b), rat carotid arteries (Gross PM *et al.*, 1981a), and rat basilar arteries (Chang JY *et al.*, 1988) and is thought to be insensitive to endothelial denudation. H<sub>2</sub> receptors are thought to be preferentially located on the vascular smooth muscle (Gross PM, 1981). It seems plausible that histamine stimulation of adenylate cyclase, via smooth muscle H<sub>2</sub>/G<sub>s</sub>, leading to increased cAMP levels, could cause the activation of PKA, resulting in the inhibition of the smooth muscle contractile machinery and, thus, vasodilation.

The dimaprit experiments proved that activation of nasal H<sub>2</sub> receptors could cause nasal blockage (although not sneezing, itching or rhinorrhea), but whether or not the H<sub>2</sub> receptor contributed to histamine-induced nasal blockage was not certain.

Figures 4.11, 4.12, 4.13, 4.15, 4.16 and 4.17 all showed that the effect of an H<sub>2</sub> antagonist (commonly ranitidine), alone, on 400µg histamine-induced nasal blockage appeared to be inconsistent and not dose-related. Figures 4.11, 4.12 and 4.13 showed no reduction in histamine-induced nasal blockage by oral ranitidine (≤150mg), whereas Figures 4.15 and 4.16 showed significant inhibition of histamine-induced nasal blockage by ranitidine, 75mg, alone. The significant effect of 75mg ranitidine in inhibiting histamine-induced nasal blockage shown in Figure 4.15 suggested that the H<sub>2</sub> component in histamine-induced nasal blockage was easier to detect in experiments with greater subject numbers. The original power calculation to provide the required n number was based on a bigger ranitidine effect than was observed. By combining all the studies which investigated the effect of ranitidine, alone, on 400µg histamine-induced nasal blockage into a meta-analysis, far greater n numbers could be incorporated into a statistical analysis than could have realistically taken part in any original study. Thus an analysis with greater subject numbers could reveal a smaller ranitidine effect. After proving that each response (AUC following histamine challenge after pretreatment with a given dose of ranitidine) was not dependent on the individual subject, i.e. the presence of the H<sub>2</sub> component was not biased by the individuals in the sampled population; the meta-analysis (Figure 4.20) showed a significant inhibition of histamine-induced nasal blockage by ranitidine alone. Therefore, histamine does cause nasal blockage by a H<sub>2</sub>-mediated mechanism. This conclusion is in agreement with other reports in the literature (Secher C *et al.*, 1982; Mygind N *et al.*, 1983), although in a report by Wood-Baker *et al.* (1996), the observed reduction of histamine-induced nasal blockage by ranitidine, 150mg, was not statistically significant.

The requirement for a large n number in order to obtain a significant ranitidine effect suggests that either the ranitidine effect is only present in some individuals or the ranitidine effect is very small. H<sub>2</sub> receptor polymorphisms have been identified in man but these have, as yet, not revealed any functional changes in H<sub>2</sub> receptor phenotype (Sasaki Y *et al.*, 2000; Mancama D *et al.*, 2002). In any case, the insignificant correlation between subject and response showed that, in this study,

the effect of ranitidine on histamine-induced nasal blockage was probably not dependent on the individual subject. It seems more likely that the reason for the high n number requirement is to expose the small, but real, effect on histamine-induced nasal blockage. A small reduction of histamine-induced nasal blockage by H<sub>2</sub> antagonists is also reported by Mygind *et al.* (1983), although Secher *et al.* (1982) demonstrated that histamine-induced nasal blockage was inhibited by ranitidine and an H<sub>1</sub> antagonist equally.

Figure 4.20 also showed that this sensitivity of 400µg histamine-induced nasal blockage to ranitidine was not altered by increasing the antagonist dose from 32.5 to 300mg. This suggests that either the H<sub>2</sub> component of histamine-induced nasal blockage is not dose-dependent or that the maximally effect dose of ranitidine for 400µg histamine is ≤32.5mg. The standard clinical dose for ranitidine (when prescribed to treat excessive gastric acid secretion) is 150mg twice a day. 32.5mg ranitidine causes a plasma concentration of ~280nM, 2 hours after oral administration (McNeil JJ *et al.*, 1981). Assuming the Law of Mass Action applies and given the dissociation equilibrium constant of ranitidine at the H<sub>2</sub> receptor (~63nM), ranitidine at 32.5mg occupies ~82% of H<sub>2</sub> receptors. In comparison with endogenous histamine-stimulation of gastric acid release, a ranitidine plasma concentration of 1060nM (94% occupation of H<sub>2</sub> receptors) is the maximally effective dose in reducing acid secretion (Hirschowitz BI *et al.*, 1982). A plasma concentration of 280nM reduces gastric acid release by ~50% (Holloway RH *et al.*, 1984). Thus, for the inhibition of endogenous histamine at gastric H<sub>2</sub> receptors, a dose of 32.5mg is much lower than the maximal effective dose. It seems unlikely, therefore, that occupation of 82% of nasal H<sub>2</sub> receptors by ranitidine, 32.5mg, is sufficient to fully inhibit all the histamine-induced H<sub>2</sub>-mediated nasal blockage, unless the concentration of histamine at nasal H<sub>2</sub> receptors is low.

It is impossible to know the exact volume of distribution of exogenously administered histamine in the nasal mucosa, thus it is impossible to calculate the histamine concentration at nasal H<sub>2</sub> receptors. However, it is possible to estimate the volume of distribution as being approximately the first 2 to 3mm of the nasal mucosa (nasal symptoms of sneezing, pruritus and congestion suggest that histamine, in significant amounts, reaches mucosal blood vessels and sensory neurones) which, assuming a continuous mucosa shaped as a regular toroidal



cylinder (volume =  $\pi \cdot h \cdot (r_2^2 - r_1^2)$ ) 3cm long and with an inner radius ( $r_1$ ) of 0.5cm, would mean a volume of distribution of exogenous histamine as being approximately 3cm<sup>3</sup>. As the dose was 400µg, this means that the [histamine]<sub>nasal</sub> was approximately 400µM. In comparison with studies using exogenous histamine to stimulate gastric acid secretion this [histamine]<sub>nasal</sub> seems relatively high: [histamine]<sub>plasma</sub> of 100nM caused significant acid release and 1µM caused a maximal response (Knight SE *et al.*, 1980); and in another study, 1mmol, given subcutaneously, stimulated significant gastric acid release (Desai HG *et al.*, 1967). These comparisons are deeply flawed: rough estimation of volume of distribution; assumptions have been made on the uniformity of histamine and receptor distribution, membrane permeability to histamine, and ligand-receptor binding; and histamine metabolism and venous clearance have not been taken into account. Nevertheless, the comparisons suggest that the concentration of histamine at nasal H<sub>2</sub> receptors is not low, thus it seems unlikely that oral ranitidine, 32.5mg, would be a maximally effective dose. It is possible that an oral dose of 32.5mg is not the maximally effective dose but because the effect of H<sub>2</sub> antagonism on histamine-induced nasal blockage is minor, it is very difficult to elucidate any dose-dependence.

A combined pretreatment of ranitidine and cetirizine, the H<sub>1</sub> antagonist, caused a greater inhibition of histamine-induced nasal blockage than cetirizine alone in all the relevant studies (Figures 4.14, 4.15 and 4.16), which is in agreement with the available literature (Secher C *et al.*, 1982; Havas TE *et al.*, 1986; Wood-Baker R *et al.*, 1996). In fact, in one study (Figure 4.13), neither ranitidine, 150mg, nor cetirizine, 5mg, when administered alone, had any effect; but in combination, significantly reduced 400µg histamine-induced nasal blockage.

Interestingly, the greater inhibition of histamine-induced nasal blockage by the combination of cetirizine and ranitidine compared to cetirizine alone was consistently significant. It is possible that the H<sub>2</sub> component of histamine-induced nasal blockage is easier to demonstrate once the H<sub>1</sub> component is inhibited. Although this has not previously been studied in the human nasal airways, there is some evidence for this in the blood vessels of other organs (Powell JR *et al.*, 1976a; Brody MJ, 1980). The data suggest that there is some degree of interaction between the H<sub>1</sub> and H<sub>2</sub> mediated pathways, a theory also suggested by Howarth *et al.* (2000). The H<sub>1</sub> receptor is thought to cause vasodilation via an endothelial-

dependent mechanism, as discussed in Chapter 3.4; and, as mentioned previously in this chapter, the H<sub>2</sub> receptor is thought to be located on the vascular smooth muscle cell membrane. If there is any interaction between H<sub>1</sub>- and H<sub>2</sub>-mediated pathways, it seems likely that this interaction would occur downstream of both receptors, i.e. in the smooth muscle cell, possibly involving inhibitors of the contractile machinery, such as PKA and PKG. Alternatively, the data could suggest that activation of H<sub>1</sub> receptors may inhibit the activation of H<sub>2</sub> receptors, thus when the H<sub>1</sub> receptors are antagonized the H<sub>2</sub> receptors full capacity for vasodilation is realized.

Clearly the role of H<sub>2</sub> receptors in histamine-induced nasal blockage is not as obvious as the role of H<sub>1</sub> receptors. The meta-analysis suggests that H<sub>2</sub> receptors do contribute to histamine-induced nasal blockage but the other data indicate further complexity. One particularly puzzling result, as shown in Figure 4.16, was that a combination of cetirizine and ranitidine was more effective in inhibiting nasal blockage compared to cetirizine alone but not ranitidine alone. There seems to be no reasonable mechanism by which cetirizine, in this particular study, failed to further reduce nasal blockage when in combination with ranitidine. However, given the high number of studies and statistical tests on the effect of ranitidine on nasal blockage in this chapter, it is possible that type 1 (false positive) and type 2 (unnoticed positive) errors have occurred, i.e. the unexpected can always occur by chance.

As shown by Figures 4.15 and 4.17, a combination of maximally effective doses of both cetirizine (H<sub>1</sub> antagonist) and ranitidine (H<sub>2</sub> antagonist) failed to fully abolish 400µg histamine-induced nasal blockage, although in Figure 4.13 there was no significant difference between the nasal patency in the saline control group and the histamine after pretreatment with antagonist combination group. Therefore, although both H<sub>1</sub> and H<sub>2</sub> pathways contribute to histamine-induced nasal blockage; it appears another, non-H<sub>1</sub>/H<sub>2</sub>, pathway is responsible for the residual nasal blockage, a theory also suggested by Howarth *et al.* (2000).

#### 4.5 Summary

- 1) Dimaprit (H<sub>2</sub> agonist), 200µg, induced a significant decrease in the patency of the nasal airways of normal individuals, observed as an increase in nasal blockage.
- 2) Pretreatment with orally administered ranitidine (H<sub>2</sub> antagonist) abolished the dimaprit-induced nasal blockage.
- 3) Pretreatment with orally administered ranitidine significantly reduced histamine-induced nasal blockage.
- 4) Pretreatment with a combination of cetirizine (H<sub>1</sub> antagonist) and ranitidine significantly reduced histamine-induced nasal blockage as compared to either antagonist alone.
- 5) Following pretreatment with a combination of maximally effective doses of cetirizine and ranitidine, histamine frequently causes a significant nasal blockage.
- 6) The data indicate that histamine causes nasal blockage in normal individuals via H<sub>1</sub> and H<sub>2</sub> receptors in addition to, at least, one other mechanism.

## CHAPTER 5

# THE ROLES OF THE HISTAMINE H<sub>1</sub> RECEPTOR AND HISTAMINE H<sub>2</sub> RECEPTOR IN POLLEN-INDUCED NASAL RESPONSES

### 5.1 Introduction

Although hay fever had been reported in Europe since the 16<sup>th</sup> century, it was not until 1873, when Charles Blackley established that administration of pollen to the nasal mucosa caused the symptoms of the disease, that pollen was recognized as being responsible for hay fever (Varney V, 1991). Nowadays, recombinant DNA technology has provided molecular characterization of many of the most common seasonal allergens (Deinhofer K *et al.*, 2004) and it is known that pollen causes the symptoms of seasonal allergic rhinitis (hay fever) by binding and cross-linking IgE on the surface of nasal mast cells, thus stimulating mast cell degranulation (see Chapter 1.3.2).

Activation and degranulation of nasal mast cells leads to the release of many inflammatory mediators such as prostaglandins, leukotrienes, kinins and histamine. Histamine is thought to play a significant role in the pathology of seasonal allergic rhinitis: histamine levels increase in nasal lavages following allergen challenge (Mygind N, 1982; Naclerio RM *et al.*, 1985); and mRNA levels for H<sub>1</sub> receptors, H<sub>2</sub> receptors and histidine decarboxylase are higher in atopic individuals (Iriyoshi N *et al.*, 1996; Hirata N *et al.*, 1999a; Hirata N *et al.*, 1999b). In addition, when administered to the nasal mucosal, histamine causes symptoms similar to pollen: nasal congestion, rhinorrhea, pruritus and sneezing (Doyle WJ *et al.*, 1990; Howarth PH *et al.*, 2000). In Chapters 3 and 4, histamine-induced nasal blockage was shown to be reduced by both H<sub>1</sub> and H<sub>2</sub> antagonists, thus indicating that histamine-induced nasal blockage was mediated by both H<sub>1</sub> and H<sub>2</sub> receptors. In this chapter, the effect of cetirizine, an H<sub>1</sub> antagonist, and ranitidine, an H<sub>2</sub> antagonist, on pollen-induced symptoms was investigated.

Cetirizine is highly selective for H<sub>1</sub> receptors as compared to H<sub>2</sub>, H<sub>3</sub>, serotonergic, adrenergic and muscarinic receptors (Bernheim J *et al.*, 1991; Kato M *et al.*, 1997; Gillard M *et al.*, 2003). Ranitidine is highly selective for H<sub>2</sub> receptors (Mills JG *et al.*, 1989; van der Goot H *et al.*, 2000).

## **5.2 Experimental protocol**

In the following double-blind experiment, human subjects with a history of seasonal allergic rhinitis and a positive skin prick test to grass pollen were used.

### **5.2.1 The effect of the histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination with the histamine H<sub>2</sub> antagonist, ranitidine, on the nasal symptoms caused by grass pollen**

The aim of this experiment was to assess the role of H<sub>1</sub> and H<sub>2</sub> receptors in mediating the nasal symptoms following pollen challenge. The sympathomimetic, xylometazoline, was used to show that the pollen-induced nasal blockage was pharmacologically reversible. Subjects received oral cetirizine, 10mg, or oral cetirizine, 30mg, or an oral combination of cetirizine, 10mg, and ranitidine, 300mg, or oral placebo. 90 minutes later, subjects were assessed for nasal symptoms using sneeze counts; symptom scores for pruritus, rhinorrhea and congestion; and acoustic rhinometry (measures minimum cross-sectional area (A<sub>min</sub>) of the nasal cavity). Five minutes later, subjects received saline, by 100µl aerosol into each nostril. Ten and 20 minutes after saline challenge, subjects were again assessed for nasal symptoms. Five minutes later, subjects received, by 100µl aerosol into each nostril, grass pollen, 500U. Ten, 20 and 30 minutes after pollen challenge, subjects were again assessed for nasal symptoms. Five minutes later, subjects received, by 100µl aerosol into each nostril, xylometazoline (0.1% w/v). Finally, 20 minutes later, A<sub>min</sub> and congestion scores were recorded. Each subject received the 4 blinded treatments in a random order, with at least a week between each treatment. Nine subjects were included in the study. The dose of pollen was based on pilot studies and published reports (Turner *et al.*, 2001). The doses of cetirizine and ranitidine were based on previous experiments in Chapters 3 and 4 and on published reports (Holmberg K *et al.*, 1989c; Falliers CJ *et al.*, 1991; Wang D *et al.*, 1996). The protocol is summarized in Figure 5.1.

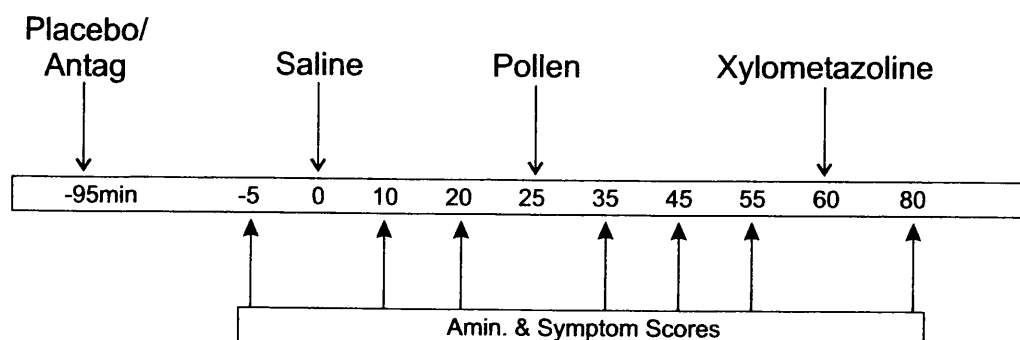


Figure 5.1: Protocol for the effect of oral histamine antagonists on the nasal symptoms caused by grass pollen. Antag = oral histamine antagonist. Note that the antagonists are administered 2 hours prior to nasal challenge with pollen.

### 5.2.2 Data Analysis

Nasal patency was measured using acoustic rhinometry, as described in Chapter 2. Subjective congestion, rhinorrhea and pruritus were separately assessed using a scoring system (0-5, 0 = no symptom, 5 = worst symptom imaginable) which was scored by the subject, as described in Chapter 2. Sneezing was assessed by counting the number of sneezes. At each time point nasal patency (Amin.), congestion, rhinorrhea, pruritus and sneezes were assessed, and plotted against time. For comparisons between the effect of pretreatment on the overall response to pollen, the data for each parameter (e.g. Amin., congestion, etc) describing the effect of pollen ( $t = 35, 45$  and  $55$  minutes) were combined into one value. For Amin., the data at  $t = 35, 45$  and  $55$  minutes were first normalised to the pre-pollen challenge value ( $t = 20$  minutes) before being plotted against time and the area under the curve (between  $t = 25$  minutes and  $t = 55$  minutes) calculated. Thus the effect of pollen at  $t = 35, 45$  and  $55$  minutes on nasal patency was combined for each treatment for each subject into one area under the normalised Amin. response versus time curve value (units: unit.min). For symptom scores, the scores for each subject at  $t = 35, 45$  and  $55$  minutes were first corrected for any measured pre-pollen challenge score by subtracting the pre-pollen score ( $t = 20$  minutes) from the post-pollen challenge scores. The corrected symptom scores were then plotted against time and the area under the curve (between  $t = 25$  minutes and  $t = 55$  minutes) calculated. Thus the effect of pollen at  $t = 35, 45$  and  $55$  minutes on each nasal symptom was combined for each treatment for each subject into one area under the corrected symptom score versus time curve value (units: unit.min). The rhinometry, symptom score and sneeze data are presented as mean values,

together with s.e.mean. The appropriate non-parametric statistical test is given with each data set.  $p < 0.05$  is taken as significant.



### 5.3 Results

#### 5.3.1 The effect of the histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination with the histamine H<sub>2</sub> antagonist, ranitidine, on the nasal symptoms caused by grass pollen

Nasal challenge with saline had no effect on nasal responses at  $t = 10$  minutes and  $t = 20$  minutes, compared with pre-saline values ( $t = -5$  minutes), as measured by acoustic rhinometry, sneeze counts and symptom scores for congestion, rhinorrhea and pruritus ( $p > 0.05$ , Wilcoxon sign-rank test) (Figures 5.2 and 5.3).

Nasal challenge with pollen (at  $t = 25$  minutes) caused a significant decrease in  $A_{min}$ , as measured by acoustic rhinometry ( $t = 35, 45$  and  $55$  minutes), compared to pre-pollen values ( $t = 20$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 5.2).

Nasal challenge with pollen (at  $t = 25$  minutes) caused a significant increase in symptom scores for congestion, rhinorrhea and pruritus ( $t = 35, 45$  and  $55$  minutes), as compared to pre-pollen values ( $t = 20$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test) (Figures 5.2 and 5.3).

Nasal challenge with pollen (at  $t = 25$  minutes) caused a significant increase in sneeze counts ( $t = 35$  minutes), as compared to pre-pollen values ( $t = 20$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 5.3).

Nasal challenge with xylometazoline (at  $t = 60$  minutes) caused a significant increase in  $A_{min}$  ( $t = 80$  minutes), as compared to pre-xylometazoline values ( $t = 55$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 5.2). In addition, there was no significant difference between the  $A_{min}$  measured before pollen challenge ( $t = 20$  minutes) and after xylometazoline challenge ( $t = 80$  minutes) ( $p > 0.05$ , Wilcoxon sign-rank test).

Nasal challenge with xylometazoline (at  $t = 60$  minutes) caused a significant decrease in congestion symptom scores ( $t = 80$  minutes), compared with pre-xylometazoline values ( $t = 55$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 5.2). In addition, there was no significant difference between the congestion scores

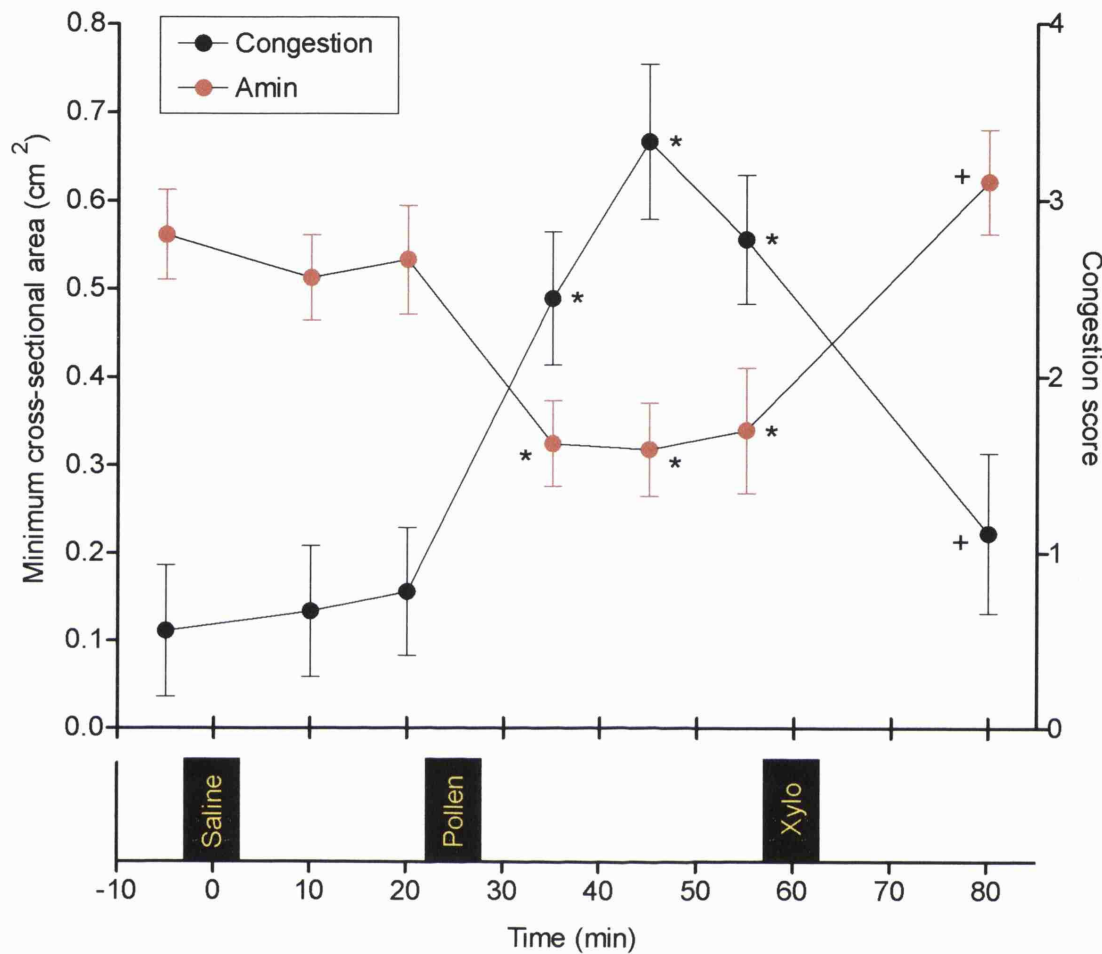


Figure 5.2: The effect of pollen, administered as an aerosol into each nostril, on the minimum cross-sectional area (Amin.) [●] and on congestion symptom scores [●]. Amin. and congestion scores were recorded at  $t = -5, 10, 20, 35, 45, 55$  and  $80$  minutes. Nasal challenge with saline, pollen (500U) or xylometazoline (0.1% w/v) [Xylo], administered as an aerosol into each nostril, was performed at  $t = 0, 25$  and  $60$  minutes, respectively. Five Amin. recordings were made for each nostril at each time-point using acoustic rhinometry and the 10 values were averaged into a single Amin. value for each time-point. Subjects graded their nasal congestion on a scale from 0 to 5 (0 = no congestion, 5 = fully congested). The data are means from 9 subjects. Vertical bars represent s.e.mean. \* Significant decrease in Amin./increase in congestion score as compared to pre-allergen challenge values ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant increase in Amin./decrease in congestion score following xylometazoline challenge as compared to pre-xylometazoline values (i.e. at  $t = 55$  minutes) ( $p < 0.05$ , Wilcoxon sign-rank test).

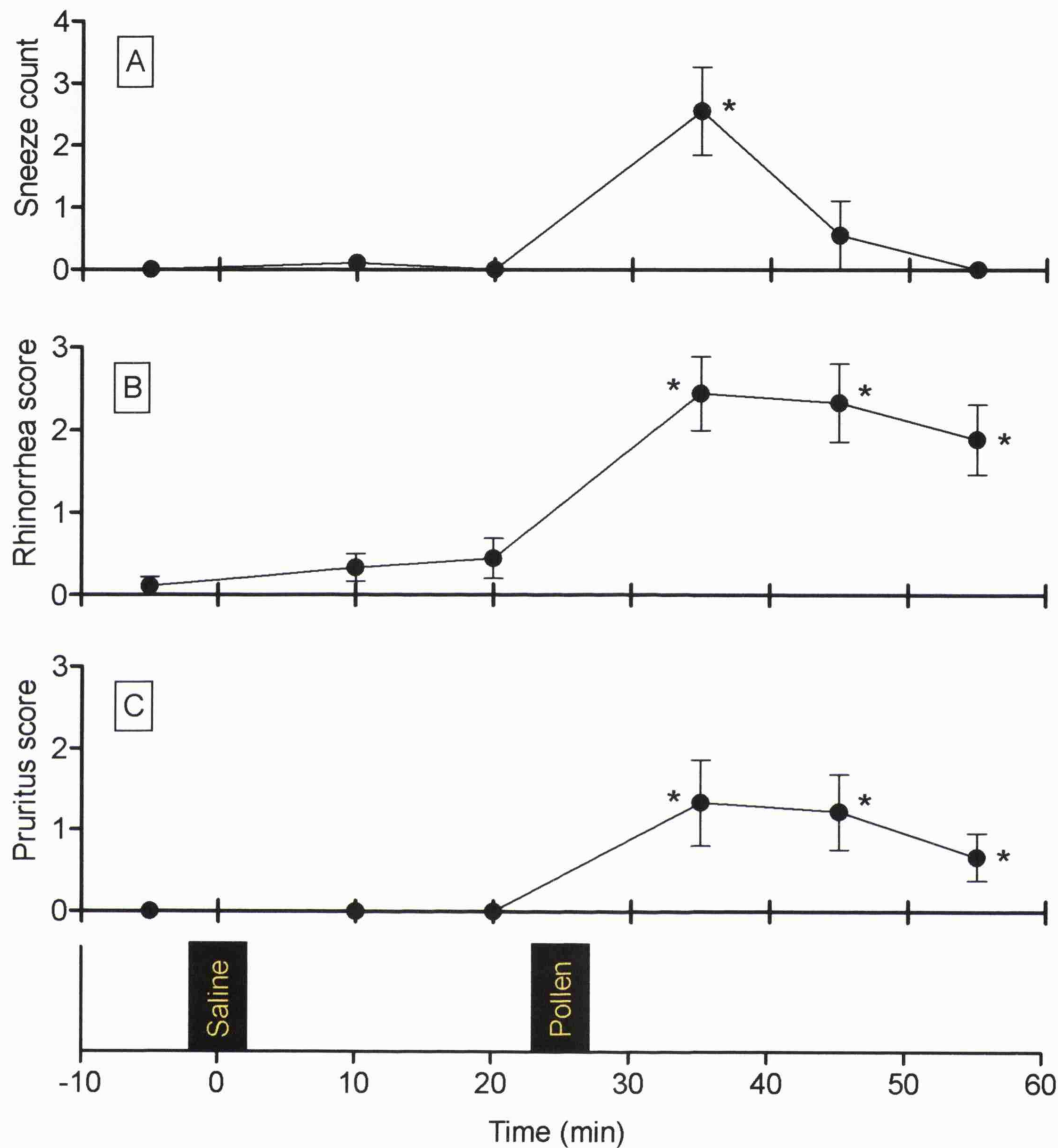


Figure 5.3: The effect of pollen, administered as an aerosol into each nostril, on symptom scores. Sneeze counts [A], rhinorrhea scores [B] and pruritus scores [C] were recorded at  $t = -5, 10, 20, 35, 45$ , and  $55$  minutes. Nasal challenge with saline or pollen ( $500U$ ), administered as an aerosol into each nostril, was performed at  $t = 0$  and  $25$  minutes, respectively. For each time-point the number of sneezes was recorded by the investigator. Subjects graded their rhinorrhea and pruritus on a scale from  $0$  to  $5$  ( $0$  = no symptom,  $5$  = worst symptom imaginable). The data are means from  $9$  subjects. Vertical bars represent s.e.mean. \* Significant increase in symptom score as compared to pre-allergen challenge values ( $p < 0.05$ , Wilcoxon sign-rank test).

recorded before pollen challenge (t = 20 minutes) and after xylometazoline challenge (t = 80 minutes) (p>0.05, Wilcoxon sign-rank test).

None of the antagonist pretreatments (cetirizine, 10mg, cetirizine, 30mg, or a combination of cetirizine, 10mg, and ranitidine, 300mg), administered at t = -95 minutes, had any significant effect on any of the parameters measured before saline challenge (t = -5 minutes) or after saline challenge (t = 10 and 20 minutes), compared with placebo pretreatment (p>0.05, Wilcoxon sign-rank test), as shown in Figures 5.4, 5.5, 5.6, 5.7 and 5.8.

None of the antagonist pretreatments (cetirizine, 10mg, cetirizine, 30mg, and a combination of cetirizine, 10mg, and ranitidine, 300mg), administered 2 hours prior to pollen challenge, had any effect on the decrease in A<sub>min</sub> caused by pollen, compared with placebo pretreatment (p>0.05, Wilcoxon sign-rank test), as shown in Figure 5.4.

Pretreatment with cetirizine, 10mg, 2 hours prior to pollen challenge, significantly reduced congestion symptom scores following pollen challenge (t = 35 and 45 minutes), compared with placebo pretreatment (p<0.05, Wilcoxon sign-rank test), as shown in Figure 5.5. None of the other pretreatments had any effect on congestion scores following pollen challenge (p>0.05, Wilcoxon sign-rank test).

None of the antagonist pretreatments (cetirizine, 10mg, cetirizine, 30mg, and a combination of cetirizine, 10mg, and ranitidine, 300mg), administered 2 hours prior to pollen challenge, had any effect on the increase in pruritus scores caused by pollen, compared with placebo pretreatment (p>0.05, Wilcoxon sign-rank test), as shown in Figure 5.6.

Pretreatment with cetirizine, 10mg, or cetirizine, 30mg, 2 hours prior to pollen challenge, significantly reduced rhinorrhea scores following pollen challenge (t = 35 minutes), compared with placebo pretreatment (p<0.05, Wilcoxon sign-rank test), as shown in Figure 5.7. Pretreatment with a combination of cetirizine, 10mg, and ranitidine, 300mg, had no effect on rhinorrhea scores following pollen challenge (p>0.05, Wilcoxon sign-rank test).

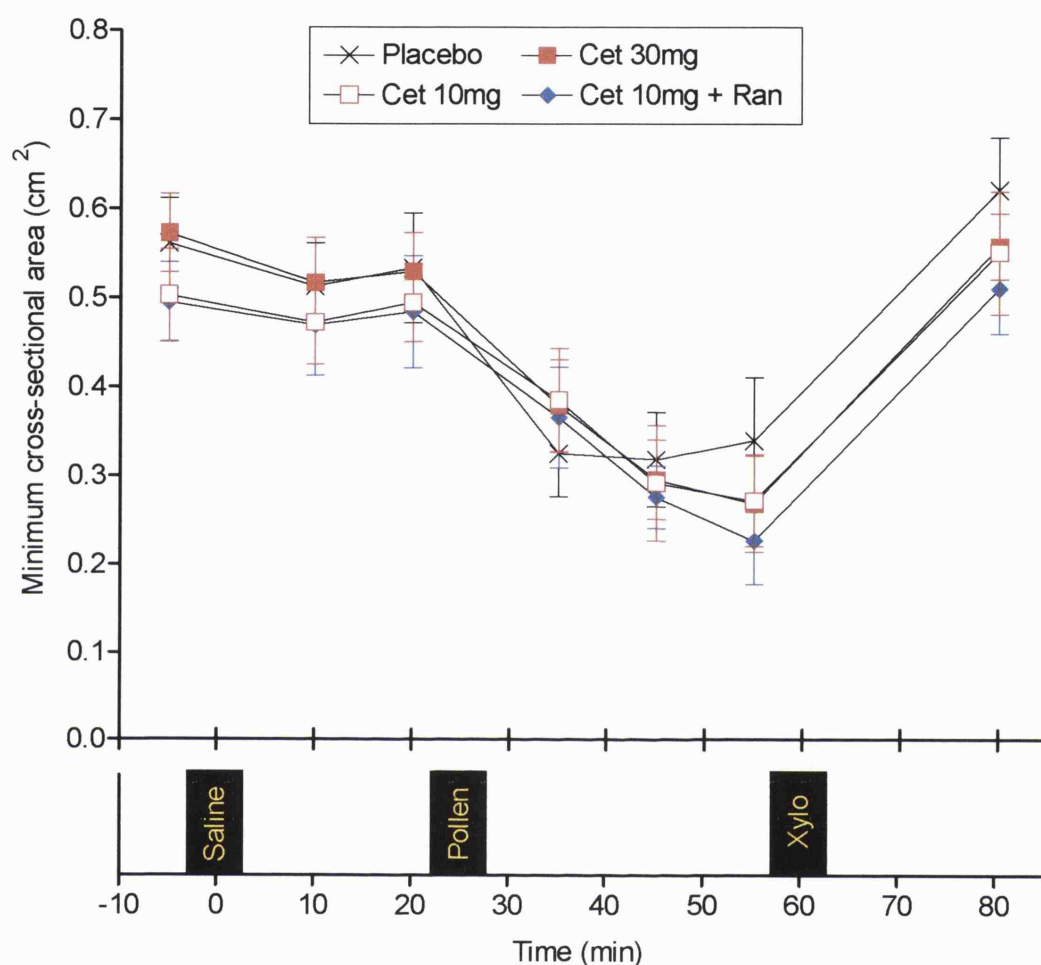


Figure 5.4: The effect of cetirizine, 10mg [□], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [◆], on the reduction of Amin. caused by pollen, 500U, administered as an aerosol into each nostril. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Amin. was recorded at t = -5, 10, 20, 35, 45, 55 and 80 minutes. Nasal challenge with saline, pollen (500U) or xylometazoline (0.1% w/v) [Xylo], administered as an aerosol into each nostril, was performed at t = 0, 25 and 60 minutes, respectively. Five Amin. recordings were made for each nostril at each time-point and the 10 values were averaged into a single Amin. value for each time-point. The data are means from 9 subjects. Vertical bars represent s.e.mean.

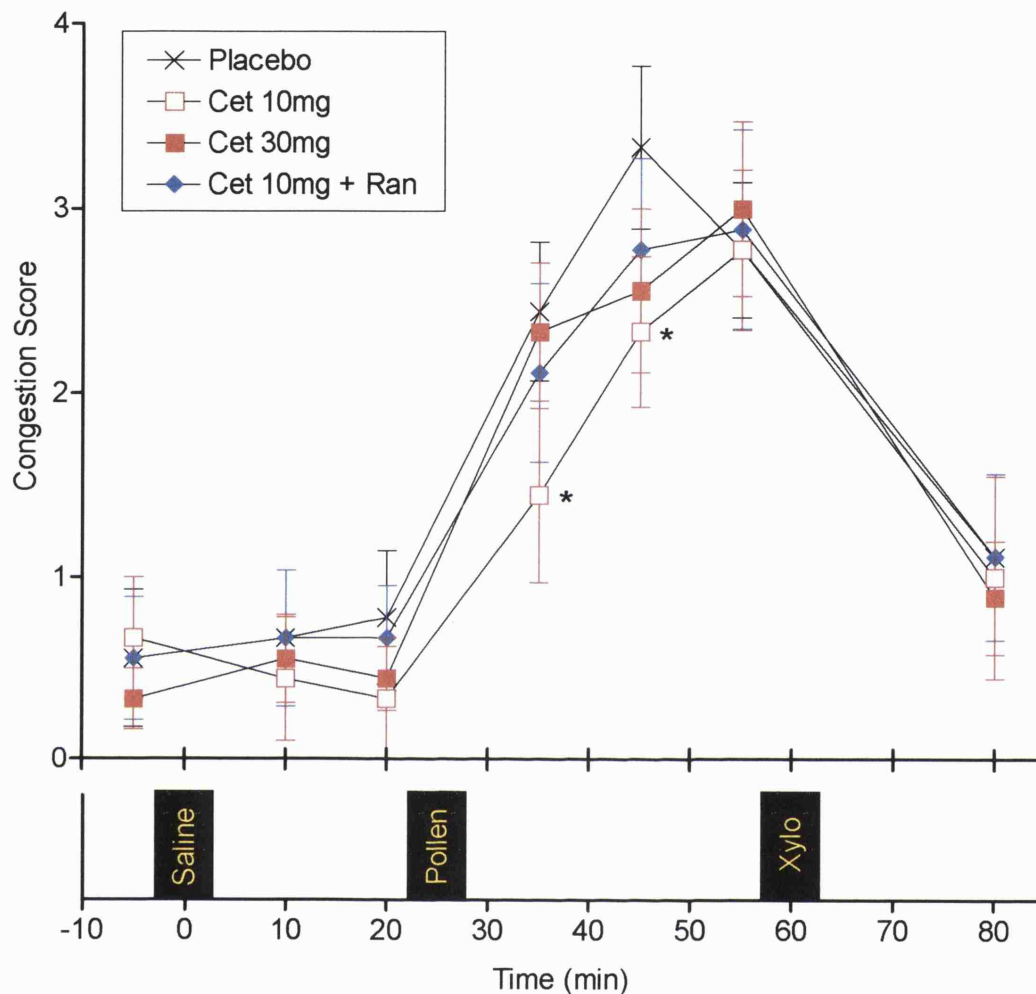


Figure 5.5: The effect of cetirizine, 10mg [□], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [◆], on the increase in congestion caused by pollen, 500U, administered as an aerosol into each nostril, as measured by congestion symptom scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Symptom scores were recorded at t = -5, 10, 20, 35, 45, 55 and 80 minutes. Nasal challenge with saline, pollen (500U) or xylometazoline (0.1% w/v) [Xylo], administered as an aerosol into each nostril, was performed at t = 0, 25 and 60 minutes, respectively. Subjects graded their nasal congestion on a scale from 0 to 5 (0 = no congestion, 5 = fully congested). The data are means from 9 subjects. Vertical bars represent s.e.mean. \* Significant reduction in nasal congestion compared with placebo pretreatment (p < 0.05, Wilcoxon sign-rank test).

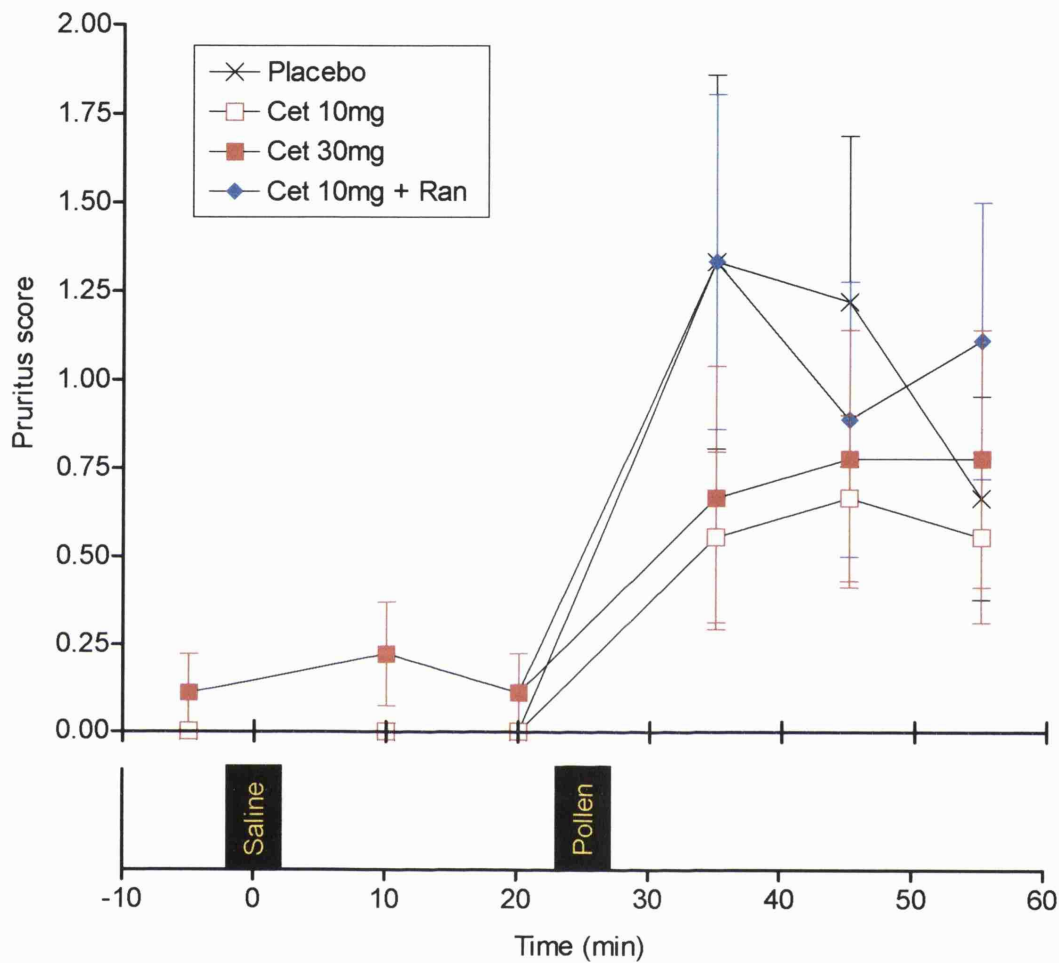


Figure 5.6: The effect of cetirizine, 10mg [□], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [◆], on the increase in pruritus caused by pollen, 500U, administered as an aerosol into each nostril, as measured by pruritus symptom scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Symptom scores were recorded at  $t = -5, 10, 20, 35, 45$  and  $55$  minutes. Nasal challenge with saline or pollen (500U), administered as an aerosol into each nostril, was performed at  $t = 0$  and  $25$  minutes, respectively. Subjects graded their nasal pruritus on a scale from 0 to 5 (0 = no pruritus, 5 = worst pruritus imaginable). The data are means from 9 subjects. Vertical bars represent s.e.mean.



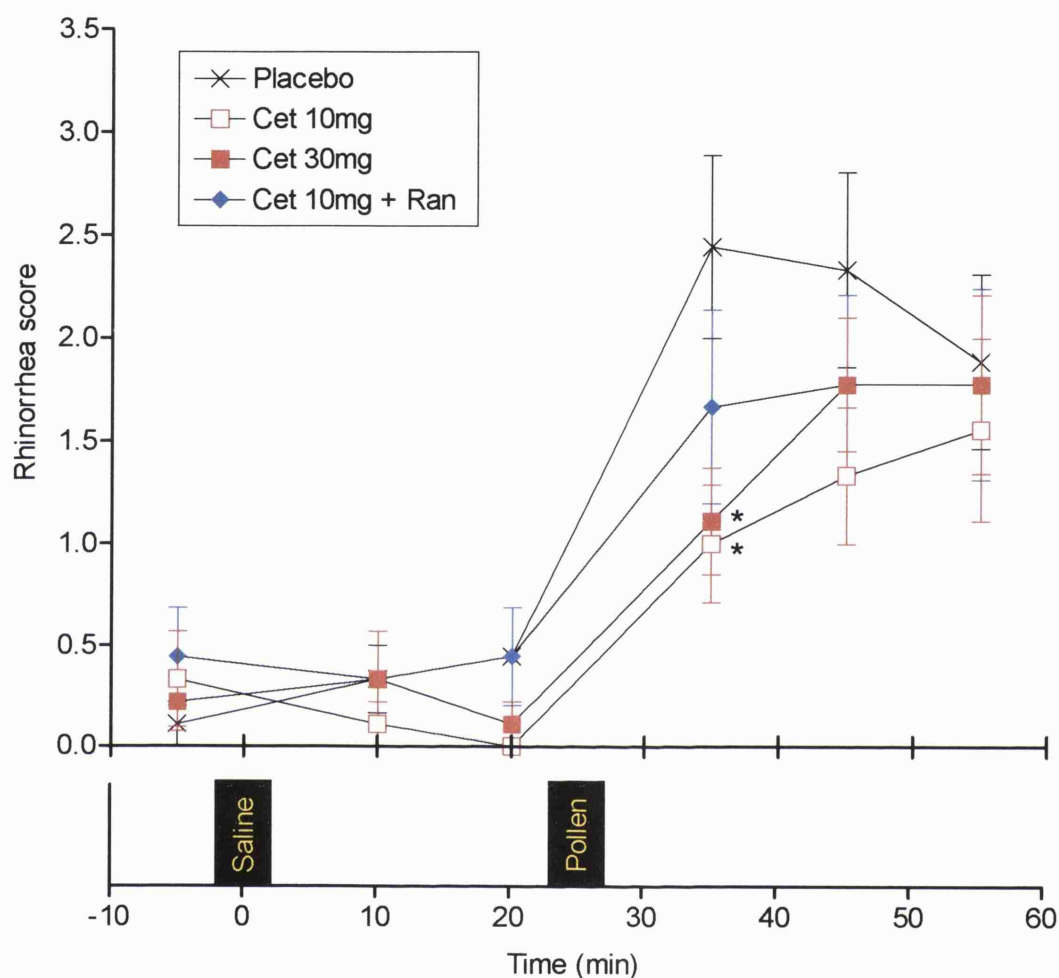


Figure 5.7: The effect of cetirizine, 10mg [□], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [◆], on the increase in rhinorrhea caused by pollen, 500U, administered as an aerosol into each nostril, as measured by rhinorrhea symptom scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Symptom scores were recorded at t = -5, 10, 20, 35, 45 and 55 minutes. Nasal challenge with saline or pollen (500U), administered as an aerosol into each nostril, was performed at t = 0 and 25 minutes, respectively. Subjects graded their rhinorrhea on a scale from 0 to 5 (0 = no rhinorrhea, 5 = worst rhinorrhea imaginable). The data are means from 9 subjects. Vertical bars represent s.e.mean. \* Significant reduction in rhinorrhea compared with placebo pretreatment (p < 0.05, Wilcoxon sign-rank test).



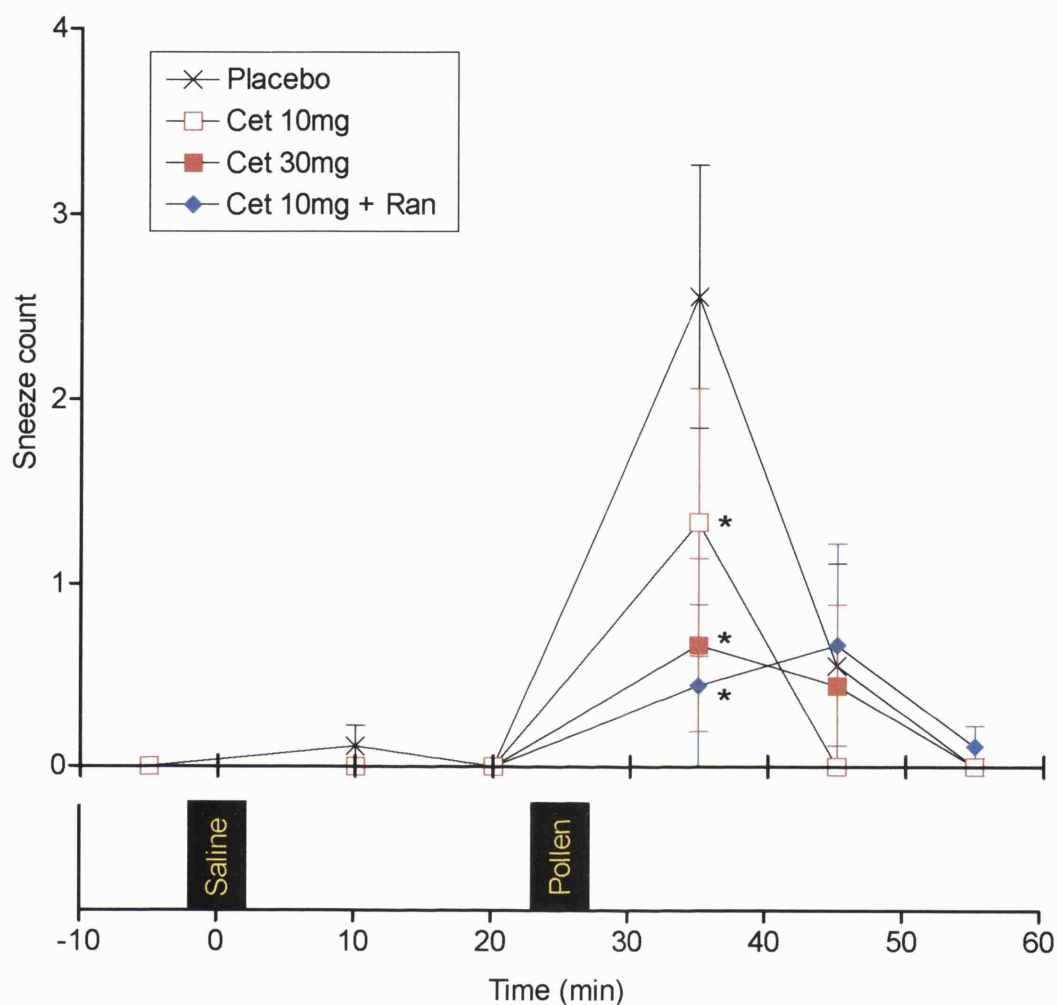


Figure 5.8: The effect of cetirizine, 10mg [□], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [◆], on the increase in sneezing caused by pollen, 500U, administered as an aerosol into each nostril, as measured by sneeze counts. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Sneezes were recorded at  $t = -5, 10, 20, 35, 45$  and  $55$  minutes. Nasal challenge with saline or pollen (500U), administered as an aerosol into each nostril, was performed at  $t = 0$  and  $25$  minutes, respectively. The data are means from 9 subjects. Vertical bars represent s.e.mean. \* Significant reduction in sneeze counts compared with placebo pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

Pretreatment with cetirizine, 10mg, or cetirizine, 30mg, or a combination of cetirizine, 10mg, and ranitidine, 300mg, 2 hours prior to pollen challenge, significantly reduced the increase in sneeze counts caused by pollen ( $t = 35$  minutes), compared with placebo pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test), as shown in Figure 5.8.

By combining the post-pollen challenge data ( $t = 35, 45$  and  $55$  minutes) into one value for each subject, it was possible to assess the effect of histamine antagonist pretreatment on the overall response to pollen. Amin. values at  $t = 35, 45$  and  $55$  minutes were normalised to the pre-pollen challenge value ( $t = 20$  minutes) for each subject. The normalised Amin. was then plotted against time (data not shown) and the area under the curve (AUC), between  $t = 25$  minutes and  $t = 55$  minutes, calculated. None of the antagonist pretreatments had any effect on the AUC following pollen challenge compared with placebo pretreatment ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 5.9).

Post-pollen challenge symptom scores ( $t = 35, 45$  and  $55$  minutes) for congestion were also combined into one value for each subject. Congestion scores at  $t = 35, 45$  and  $55$  minutes were corrected for any measured pre-pollen challenge score by subtracting the pre-pollen score ( $t = 20$  minutes) from the post-pollen challenge scores. The corrected congestion scores were then plotted against time (data not shown) and the area under the curve (between  $t = 25$  minutes and  $t = 55$  minutes) calculated. This procedure was also performed for pruritus and rhinorrhea symptom scores. None of the antagonist pretreatments had any significant effect on the AUC following pollen challenge for congestion, pruritus or rhinorrhea scores compared with placebo pretreatment ( $p > 0.05$ , Wilcoxon sign-rank test) (Figures 5.10, 5.11 and 5.12). This analysis was not performed on the sneeze count data, as sneezing was only significantly increased at one time-point following nasal challenge with pollen.

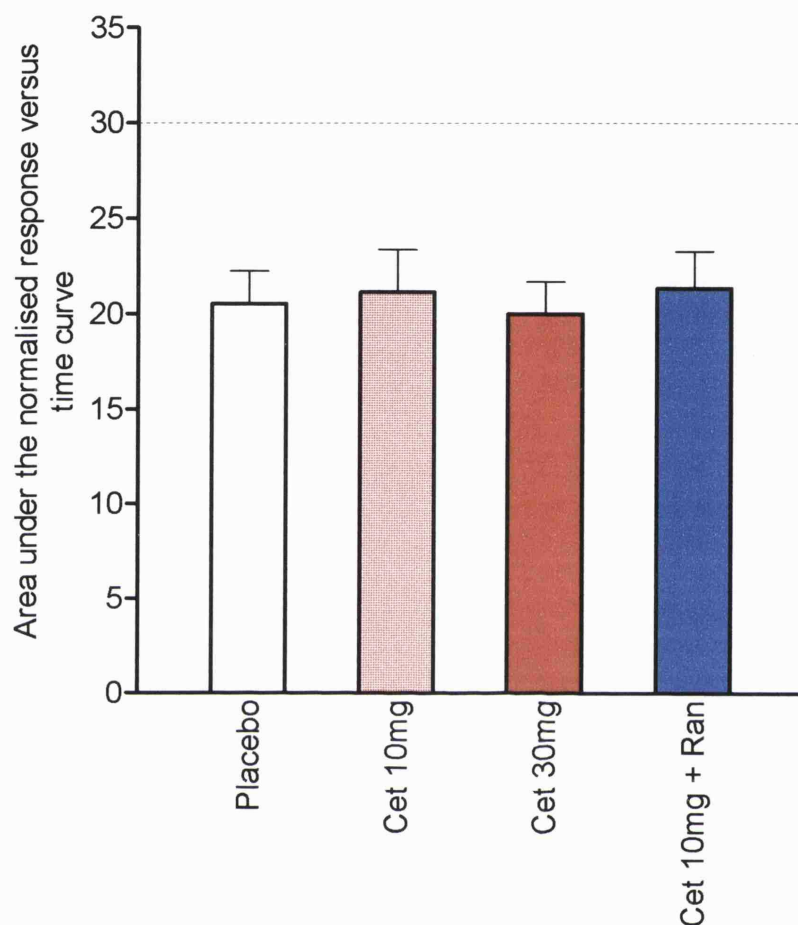


Figure 5.9: The effect of cetirizine, 10mg [■], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [■], on the nasal blockage caused by pollen, 500U, given as an aerosol into each nostril, as measured by acoustic rhinometry. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Amin. was measured 5 minutes before, and 10, 20 and 30 minutes after each nasal challenge with pollen. Amin. values were normalised to the pre-challenge value (mean of  $0.510\text{cm}^2$ , s.e.mean of  $0.025\text{cm}^2$ ). The data are means from 9 subjects and represent the area under the normalised Amin. versus time curve (AUC) measured over a 30 minute period following the nasal administration of pollen. Vertical bars represent s.e.mean. The dotted line represents the AUC measured over a 30 minute period without nasal challenge, i.e. no change in normalised Amin. over a 30 minute period following challenge would produce an AUC of 30.

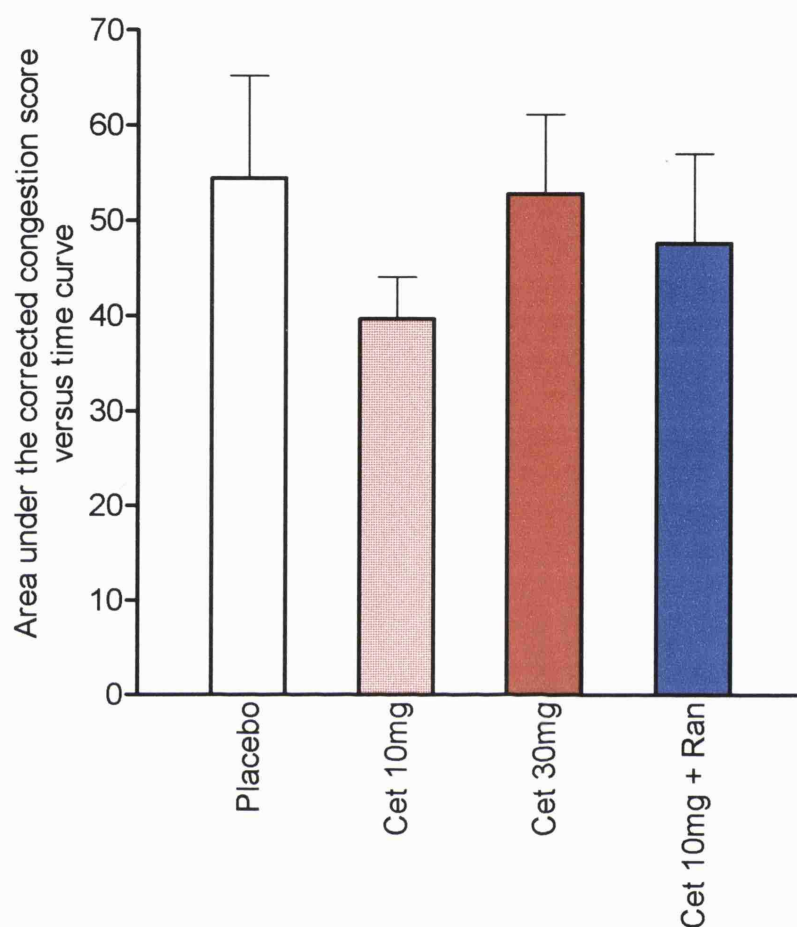


Figure 5.10: The effect of cetirizine, 10mg [■], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [■], on the nasal congestion caused by pollen, 500U, given as an aerosol into each nostril, as measured by congestion scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Congestion scores were recorded 5 minutes before, and 10, 20 and 30 minutes after each nasal challenge with pollen. Congestion scores were corrected for any measured pre-challenge value by subtracting the pre-challenge response from the recorded post-challenge responses. The corrected scores were then plotted against time and the area under the curve calculated. The data are means from 9 subjects and represent the area under the corrected congestion score versus time curve measured over a 30 minute period following the nasal administration of pollen. Vertical bars represent s.e.mean.

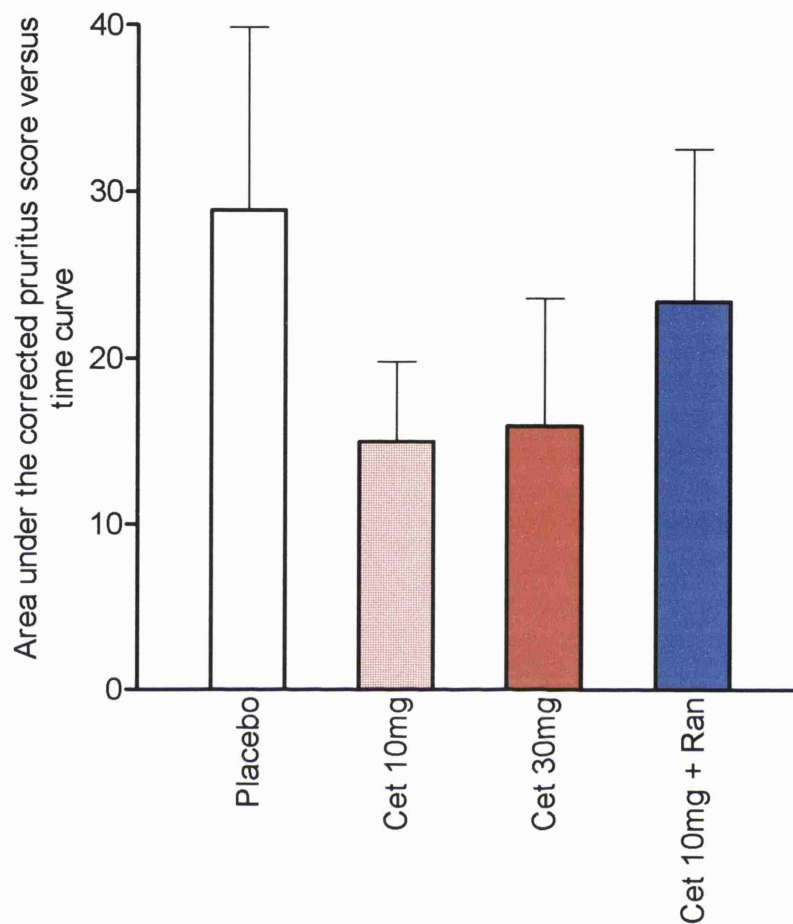


Figure 5.11: The effect of cetirizine, 10mg [■], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [■], on the nasal pruritus caused by pollen, 500U, given as an aerosol into each nostril, as measured by pruritus scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Pruritus scores were recorded 5 minutes before, and 10, 20 and 30 minutes after each nasal challenge with pollen. Pruritus scores were corrected for any measured pre-challenge value by subtracting the pre-challenge response from the recorded post-challenge responses. The corrected scores were then plotted against time and the area under the curve calculated. The data are means from 9 subjects and represent the area under the corrected pruritus score versus time curve measured over a 30 minute period following the nasal administration of pollen. Vertical bars represent s.e.mean.

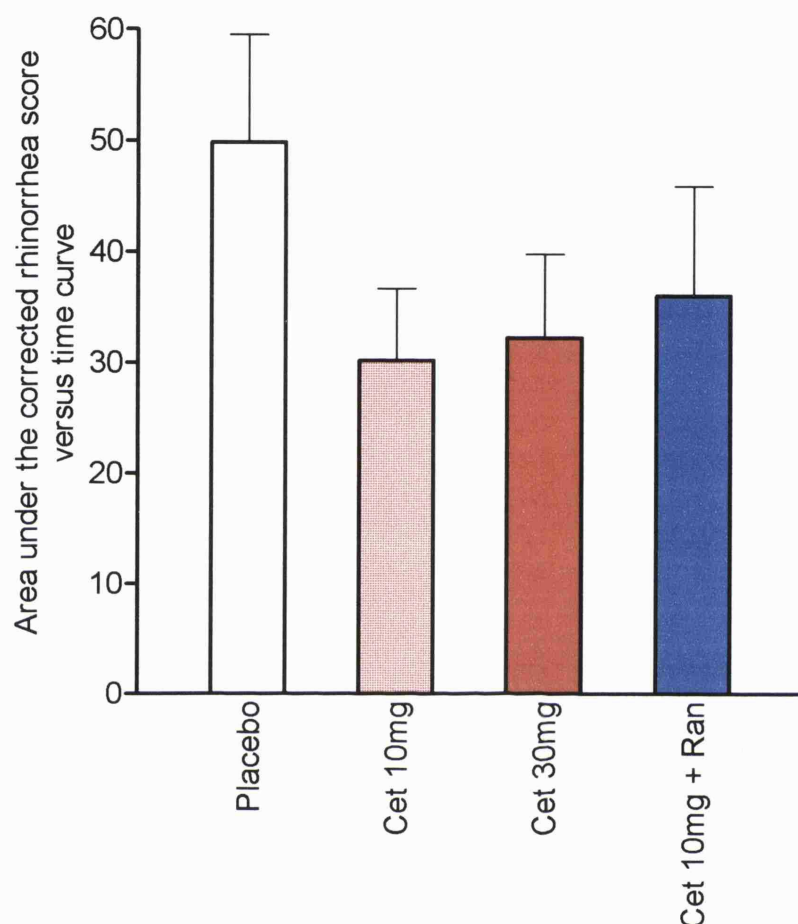


Figure 5.12: The effect of cetirizine, 10mg [■], cetirizine, 30mg [■], and a combination of cetirizine, 10mg, and ranitidine [Ran], 300mg [■], on the rhinorrhea caused by pollen, 500U, given as an aerosol into each nostril, as measured by rhinorrhea scores. Placebo, cetirizine and ranitidine pretreatments were all given orally 2 hours prior to pollen nasal challenge. Rhinorrhea scores were recorded 5 minutes before, and 10, 20 and 30 minutes after each nasal challenge with pollen. Rhinorrhea scores were corrected for any measured pre-challenge value by subtracting the pre-challenge response from the recorded post-challenge responses. The corrected scores were then plotted against time and the area under the curve calculated. The data are means from 9 subjects and represent the area under the corrected rhinorrhea score versus time curve measured over a 30 minute period following the nasal administration of pollen. Vertical bars represent s.e.mean.

## 5.4 Discussion

Pollen, 500U, caused an increase in nasal symptoms as measured by sneeze counts and symptom scores for congestion, rhinorrhea and pruritus. In addition, acoustic rhinometry measurements of the minimum nasal cross-sectional area (Amin.) showed significant reduction following pollen administration, which represents a reduction in nasal patency or an increase in nasal blockage. This is in agreement with numerous studies which showed that pollen causes nasal symptoms in sensitized individuals (Holmberg K *et al.*, 1989c; Wang D *et al.*, 1996; Wood-Baker R *et al.*, 1996).

All nasal symptoms, except sneezing, significantly persisted for the three post-pollen time-points (t = 35, 45 and 55 minutes), which is in agreement with other reports regarding the time-course of the immediate phase following allergen challenge (Naclerio RM *et al.*, 1985; Holmberg K *et al.*, 1988). Allergen-induced sneezing has previously been shown to be short-lived (Walden SM *et al.*, 1988), and this is thought to be due to tachyphylaxis of sensory neuronal mechanisms (Shelton D *et al.*, 1994).

Xylometazoline, the sympathomimetic, significantly reversed and abolished the pollen-induced nasal blockage, as measured by congestion symptom scores and acoustic rhinometry, demonstrating that the nasal blockage was not a permanent change in nasal topology.  $\alpha$ -adrenoceptor agonists, such as xylometazoline, are able to cause vasoconstriction in nasal blood vessels and have been shown in many studies to reverse allergen-induced nasal blockage (Johnson DA *et al.*, 1993; Hochban W *et al.*, 1999).

As mentioned in Chapter 1.3.2, the inflammatory mediators released after mast cell degranulation are thought to be responsible for the pathophysiological changes which lead to symptom generation. These substances promote the dilation of nasal blood vessels, causing nasal blockage and the symptom of congestion. By increasing vascular permeability and activating submucosal glands, these substances cause the symptom of rhinorrhea. Lastly, these substances activate sensory neurones, causing sneezing and pruritus.



All antagonist pretreatments (cetirizine, 10mg, cetirizine, 30mg, and a combination of cetirizine, 10mg, and ranitidine, 300mg) significantly reduced pollen-induced sneeze counts at  $t = 35$  minutes. There was no significant difference between pretreatment with cetirizine, 10mg, and the higher dose of cetirizine, 30mg, or the combination of cetirizine, 10mg, and ranitidine, 300mg. It appears that pollen-released histamine causes the symptom of sneezing via H<sub>1</sub> receptors. Pollen-induced sneezing has been shown by other studies to be reduced by H<sub>1</sub> antagonists (Holmberg K *et al.*, 1989c; Naclerio RM, 1990; Falliers CJ *et al.*, 1991; DuBuske L, 1995; Wang D *et al.*, 1996). Apart from H<sub>1</sub> antagonists, the only effective therapy so far discovered for preventing pollen-induced sneezing is topical corticosteroid treatment (Gale AE *et al.*, 1980), which also reduces histamine levels following challenge with pollen (Togias A *et al.*, 1988). Interestingly, there are no published reports of allergen-induced sneezing being inhibited by local anaesthetics, even though local anaesthetics cause nasal anaesthesia. In fact, the one study investigating local anaesthetics in treating allergic rhinitis failed to show any effect of local anaesthetics on pollen-induced symptoms, including sneezing (Holmberg K *et al.*, 1989b). There is no evidence in the data to suggest that pollen-induced sneezing is mediated via H<sub>2</sub> receptors, which is in agreement with a report by Holmberg *et al.* (1989) that failed to show any effect of cimetidine on pollen-induced sneezing. It is possible that sneezing following pollen challenge is solely mediated by histamine activating H<sub>1</sub> receptors on sensory neurones.

None of the antagonist pretreatments (cetirizine, 10mg, cetirizine, 30mg, and a combination of cetirizine, 10mg, and ranitidine, 300mg) had any significant effect on pollen-induced pruritus at the individual time points ( $t = 35, 45$  and  $55$  minutes) (Figure 5.6) and only cetirizine alone, 10 or 30mg, reduced pollen-induced rhinorrhea; and this solely at  $t = 35$  minutes (Figure 5.7). However, although none of the pretreatments had any significant effect on the overall pollen-induced pruritus (Figure 5.11) and rhinorrhea (Figure 5.12), there appears to be a trend for the histamine antagonists to reduce these two symptoms. Symptom scores tend to produce data with relatively large coefficients of variation, and the power calculation for this experiment was based upon expected changes in A<sub>min</sub> measurements (which tend to have smaller coefficients of variation). Thus it is perhaps not surprising that the number of subjects may be too low to reach significance for the effect of these antagonists on symptom scores. From the data, it appears that



pollen-released histamine may cause the symptoms of pruritus and rhinorrhea via H<sub>1</sub> receptors. H<sub>1</sub> antagonists have been shown to reduce pollen-induced pruritus and rhinorrhea in numerous other studies (Holmberg K *et al.*, 1989c; Falliers CJ *et al.*, 1991; DuBuske L, 1995; Wang D *et al.*, 1996; Wilson AM *et al.*, 2002). It is thought that histamine mainly causes H<sub>1</sub>-mediated rhinorrhea indirectly via the activation of central reflexes, leading to an increase in parasympathetic activity. Thus, histamine-induced rhinorrhea is reduced by oxitropium, a muscarinic antagonist (Birchall MA *et al.*, 1996). There appears to be no contribution of H<sub>2</sub> receptors to pollen-induced pruritus and rhinorrhea as the addition of ranitidine, 300mg, to cetirizine, 10mg, produced no traceable reduction in symptoms compared to cetirizine, 10mg, alone. There are no reports of H<sub>2</sub> antagonists reducing pollen-induced pruritus. However, one study showed a reduction of pollen-induced rhinorrhea by a topical ranitidine spray (25mg) (Holmberg K *et al.*, 1989c). It is conceivable that H<sub>2</sub> receptors play a role in pollen-induced rhinorrhea as H<sub>2</sub> receptor mRNA has been found in nasal submucosal glands (Hirata N *et al.*, 1999a). However, nasal challenge with impromidine, the H<sub>2</sub> agonist, failed to cause rhinorrhea at doses which caused nasal blockage (Shelton D *et al.*, 1994).

Cetirizine, 10mg and 30mg, had no effect on pollen-induced nasal blockage, either at individual time points ( $t = 35, 45$  or  $55$  minutes) (Figure 5.4) or over the entire response (Figure 5.9) as measured by acoustic rhinometry. In addition, a combination of cetirizine, 10mg, and ranitidine, 300mg, also had no effect on pollen-induced nasal blockage. Either pollen-released histamine does not cause nasal blockage via H<sub>1</sub> or H<sub>2</sub> receptors; or the effect of H<sub>1</sub> and H<sub>2</sub> antagonism is too small to detect; or the doses of the antagonist are not high enough.

Although cetirizine, 10mg, inhibited the pollen-induced increase in nasal congestion at  $t = 35$  and  $45$  minutes, neither cetirizine, 30mg, nor the combination of cetirizine, 10mg, and ranitidine, 300mg, had any effect on nasal congestion at these time points (Figure 5.5). In addition, none of the antagonist pretreatments had any effect on the overall pollen-induced nasal congestion, as shown in Figure 5.10. It is plausible that the significant effects of cetirizine, 10mg, at  $t = 35$  and  $45$  minutes are both type 1 errors. Thus it seems likely that either pollen-released histamine does not cause the symptom of congestion via H<sub>1</sub> or H<sub>2</sub> receptors; or the effect of H<sub>1</sub> and

H<sub>2</sub> antagonism is too small to detect; or the doses of the antagonists are not high enough.

If H<sub>1</sub> receptors are responsible for mediating nasal blockage and the symptom of congestion, it is puzzling why 30mg cetirizine has no effect on nasal blockage, whereas 10mg is sufficient to reduce pollen-induced sneezing. It seems unlikely that the concentration of pollen-released histamine would differ wildly between blood vessels and neurones in the nasal mucosa, given their similar proximity to nasal mast cells. Perhaps there are more 'spare receptors' in the H<sub>1</sub> population on blood vessels than on sensory neurones, thus even the higher occupation of vascular H<sub>1</sub> receptors by cetirizine, 30mg, may have less inhibitory effect than occupation of neuronal H<sub>1</sub> receptors by cetirizine, 10mg. Another possibility is that the H<sub>1</sub> receptors on blood vessels and sensory neurones are pharmacologically distinct, either structurally or functionally. H<sub>1</sub> receptor heterology has not been widely reported, however one study showed pharmacologically distinct populations of human H<sub>1</sub> receptors following their expression in Chinese hamster ovaries (Booth RG *et al.*, 2002). Although there are no published studies of the structure of nasal neuronal H<sub>1</sub> receptors, cetirizine (at similar doses) has previously been shown to reduce both histamine-induced nasal blockage and sneezing (Hilberg O *et al.*, 1995; Wang DY *et al.*, 2001), therefore it seems unlikely that pollen-released histamine activates cetirizine-insensitive vascular H<sub>1</sub> receptors to produce nasal blockage.

As mentioned in Chapter 1.6.3, the role of H<sub>1</sub> receptors in mediating pollen-induced nasal blockage is controversial. Most reports in the literature fail to show sensitivity of pollen-induced nasal blockage to H<sub>1</sub> antagonists (Holmberg K *et al.*, 1989c; Naclerio RM, 1990; Bousquet J *et al.*, 1990; Varney V, 1991; Falliers CJ *et al.*, 1991; Hilberg O *et al.*, 1995; Wood-Baker R *et al.*, 1996; Corren J *et al.*, 1999), although other authors have demonstrated, especially with desloratadine, a reduction of pollen-induced nasal blockage by H<sub>1</sub> antagonists (Nayak AS *et al.*, 2001; Horak *et al.*, 2002; Schenkel E *et al.*, 2002; Wilson AM *et al.*, 2002). Both desloratadine and cetirizine are potent H<sub>1</sub> antagonists, with dissociation equilibrium constants at human H<sub>1</sub> receptors of 1nM and 10nM, respectively. The maximum H<sub>1</sub> receptor occupancy following pretreatment with the standard dose of desloratadine (5mg), given its published oral bioavailability (Gupta S *et al.*, 2002), is >99%, which

is identical to the calculated maximum H<sub>1</sub> receptor occupancy of cetirizine, 30mg – the dose used in the present study. Interestingly, desloratadine, compared to other H<sub>1</sub> antagonists such as cetirizine and fexofenadine, is relatively unselective for H<sub>1</sub> receptors: for example, the selectivity ratios between human muscarinic receptors and human H<sub>1</sub> receptors for desloratadine are approximately 50 to 125, compared to >20,000 for cetirizine (Gillard M *et al.*, 2003). It is possible that the decongestant effect of desloratadine may not result from antagonism of histamine at H<sub>1</sub> receptors but by some other mechanism.

In 1981, Paul Gross hypothesized that H<sub>2</sub> receptors, preferentially located on the outer layers of the vascular smooth muscle, mediated mast cell-derived histamine-induced vasodilation; whereas endothelial H<sub>1</sub> receptors mediated vasodilation caused by intra-arterially administered histamine. It is possible that pollen-induced nasal blockage is mediated by these H<sub>2</sub> receptors and the ineffectiveness of ranitidine, 300mg, is simply the result of the high levels of histamine released by nasal mast cells. However, as shown in Chapter 3, exogenously administered histamine can be significantly reduced by oral and topical H<sub>1</sub> antagonists, indicating a role of H<sub>1</sub> receptors in mediating non-intra-arterially administered histamine-induced vasodilation. In addition, it should be remembered that pollen-induced sneezing was significantly reduced by an oral dose of cetirizine, 10mg, which, according to published plasma levels following oral cetirizine administration (Urien S *et al.*, 1999) and assuming the Law of Mass Action applies, occupies approximately 98% of H<sub>1</sub> receptors. According to the published ranitidine plasma levels following 300mg oral dose (Flores Perez J *et al.*, 2003) and assuming the Law of Mass Action applies, ranitidine, 300mg, also occupies approximately 98% of H<sub>2</sub> receptors. Although a direct comparison between neuronal H<sub>1</sub> receptors and vascular H<sub>2</sub> receptors is tenuous, it seems likely that if pollen-induced nasal blockage *is* mediated, to a significant degree, by H<sub>2</sub> receptors, ranitidine, 300mg, would reduce it to some extent. Thus, it appears unlikely that pollen-induced nasal blockage is significantly mediated by H<sub>2</sub> receptors. H<sub>2</sub> antagonists have also failed to reduced pollen-induced nasal blockage in other published reports (Holmberg K *et al.*, 1989c; Atkinson TP *et al.*, 1995).

Histamine levels in nasal lavages rise following pollen challenge (Enerback L *et al.*, 1986; Naclerio RM *et al.*, 1985). According to the data in the present study (which is

in agreement with the available literature), histamine plays a significant role in mediating pollen-induced sneezing via H<sub>1</sub> receptors. Comparing the mean sneeze count following pollen challenge, with the mean sneeze count following exogenously administered histamine (see Chapter 3.3.1); pollen, 500U, causes the same number of sneezes as histamine, 100µg. Histamine, 100µg, as shown in Chapter 3.3.1, causes significant nasal blockage when applied topically to the nasal mucosa. It seems reasonable to expect this level of pollen-induced histamine to be responsible for some of the nasal blockage. However, it appears that antagonism at H<sub>1</sub> and H<sub>2</sub> receptors is unable to reduce pollen-induced nasal blockage. Perhaps, pollen-induced histamine causes nasal blockage via a non-H<sub>1</sub>-, non-H<sub>2</sub>-mediated mechanism.

## 5.5 Summary

- 1) Pollen, 500U, induced a significant increase in nasal symptoms in atopic individuals, observed as an increase in sneeze counts and symptom scores for rhinorrhea, pruritus and congestion.
- 2) Pollen, 500U, induced a significant decrease in the patency of the nasal airways of atopic individuals, observed as an increase in nasal blockage. This was reversed by the sympathomimetic, xylometazoline.
- 3) Pretreatment with cetirizine, 10mg, significantly reduced the sneezing caused by pollen, 500U.
- 4) There was a trend for the reduction in pollen-induced pruritus and rhinorrhea following pretreatment with cetirizine.
- 5) Pretreatment with cetirizine, 10mg, or cetirizine, 30mg, or a combination of cetirizine, 10mg, and ranitidine, 300mg, had no significant effect on the pollen-induced congestion (symptom score) or the pollen-induced nasal blockage (acoustic rhinometry).
- 6) The data indicate that pollen-induced sneezing, and possibly pruritus and rhinorrhea, is mediated via histamine activation of H<sub>1</sub> receptors. Pollen-induced nasal blockage does not appear to be mediated by H<sub>1</sub> or H<sub>2</sub> receptors.

# CHAPTER 6

## THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE CONTROL OF NASAL PATENCY

### 6.1 Introduction

The nasal mucosa is densely innervated with sensory and autonomic neurones (Baraniuk JN, 1998). Sensory neurones are responsible for conducting the sensory impulses that lead to the reflex symptoms of allergic rhinitis (sneezing and pruritus), as well as stimulating centrally-reflex-mediated rhinorrhea (Baraniuk JN, 1997). In addition, activated sensory neurones release vasoactive neuropeptides, such as substance P, CGRP and NKA. However, neuropeptides have been shown not to play a significant role in controlling the nasal mucosa under resting conditions (Turner PJ, 1999) and their role in allergic rhinitis is unclear (Holmberg K *et al.*, 1989b; Cheung D *et al.*, 1994; Chiba Y *et al.*, 1994; Turner PJ, 1999). Parasympathetic neurones mainly innervate submucosal glands. Following the stimulation of central reflexes, nasal parasympathetic neurones release ACh onto submucosal glands, which results in the release of mucus (containing lactoferrin, lysozyme, neutral endopeptidase, secretory IgA and glycoproteins) (Okayama M *et al.*, 1993; Baraniuk JN, 1998). Allergen-induced rhinorrhea has been shown to be reduced by muscarinic antagonists (Baroody FM *et al.*, 1994; Kaiser HB *et al.*, 1995).

Allergen-induced nasal blockage can be reversed by systemic or topical administration of sympathomimetics (Johnson DA *et al.*, 1993; Hochban W *et al.*, 1999).  $\alpha$ -adrenoceptor agonists, such as oxymetazoline, xylometazoline and phenylephrine, activate  $\alpha$ -adrenoceptors located on nasal blood vessels (van Megan YJ *et al.*, 1991), causing vasoconstriction. This vasoconstriction reduces the swelling of the nasal turbinates and thus results in decongestion.

Despite the successful use of sympathomimetics in allergic rhinitis and the abundance of sympathetic innervation of nasal blood vessels (Baraniuk JN, 1998),

there is little direct evidence of the sympathetic playing an active role in allergic rhinitis. However, as mentioned in Chapter 1.7.3, there is a wealth of indirect evidence supporting a role of nasal sympathetic neurones in the control of resting nasal patency: nasal blockage is a side-effect following treatment for hypertension and benign prostatic obstruction by pharmacological blockade of noradrenaline (NA) release and  $\alpha$ -adrenoceptor antagonism (Moyer JH *et al.*, 1953; Ferguson RK *et al.*, 1976; Le Gras MD *et al.*, 1990). In addition, sympathectomy causes nasal blockage in man (Whittet HB *et al.*, 1988); and increased sympathetic activity, whether acutely as during exercise (Konno A *et al.*, 1982; Juto JE *et al.*, 1984), or periodically due to the hypothalamic-mediated nasal cycle (Kennedy B *et al.*, 1986; Hanif J *et al.*, 2000), causes nasal decongestion. Nevertheless, there are no hypothesis-tested studies published in the literature which confirm an active role of the sympathetic nervous system in the control of nasal patency in the resting nose. In this chapter, the role of nasal sympathetic neurones in the control of nasal patency has been investigated.

Nasal sympathetic neurones contain either NA and NPY or NA alone (Baraniuk JN, 1998). Both NA and NPY are potent vasoconstrictors (Wang SH *et al.*, 1993; Baraniuk JN, 1998). However, given that the evidence suggests  $\alpha$ -adrenoceptors mediate the majority of sympathetic-induced decongestion and the pharmacological tools for investigating NPY are limited, it seems logical to concentrate on NA as the important sympathetic-derived mediator in this system.

NA has been measured using fluorescence and electrochemical high pressure liquid chromatography (HPLC-ECD) in nasal biopsy samples from pigs, rabbits, dogs and guinea-pigs (Arihood SA *et al.*, 1976; Kubo N *et al.*, 1989a; Stjorne P, 1991). Unfortunately, no NA measurements in nasal biopsies in man have been published. In addition, no attempt has been made to measure NA in nasal lavages. If NA is responsible for maintaining a degree of nasal decongestion in the resting nose, it might be possible to measure NA (or its metabolites) in nasal lavages and to alter nasal patency using drugs which interfere with sympathetic/NA function.

NA synthesis occurs in the cytosol of sympathetic nerve terminals. Tyrosine is converted by tyrosine hydroxylase into dihydroxyphenylalanine (DOPA), which, in turn, is converted into dopamine (by DOPA decarboxylase) and then into NA by

dopamine- $\beta$ -hydroxylase (van Euler US, 1972). Tyrosine hydroxylase has been found in human nasal sympathetic neurones using immunohistochemistry (Riederer A *et al.*, 1996; Tasman AJ *et al.*, 1998). Dopamine- $\beta$ -hydroxylase, thought to be located actually inside NA secretory vesicles, has also been shown in human nasal sympathetic neurones using immunohistochemistry (Chen Y *et al.*, 1993). Newly synthesized NA and NA absorbed from the synaptic cleft is transported into secretory vesicles (by monoamine vesicle transporters) where it remains until the vesicle, in response to presynaptic depolarization, fuses with the plasma membrane and releases its tightly-packed contents into the synapse. Depolarization of the nerve terminal causes the opening of N-type voltage-operated  $\text{Ca}^{2+}$  channels (VOCC), which leads to an influx of  $\text{Ca}^{2+}$ . A high  $[\text{Ca}^{2+}]_{\text{intracellular}}$  activates vesicle exocytosis, and therefore stimulates NA release. VOCC are indirectly inhibited by the activation of presynaptic  $\alpha_2$ -adrenoceptors.  $\alpha_2$ -adrenoceptors, coupled to  $G_i$ , inhibit the formation of cAMP by adenylate cyclase. cAMP promotes the opening of VOCC. Therefore, there exists a negative feedback mechanism by which release NA inhibits cAMP, thereby reducing VOCC activation and  $\text{Ca}^{2+}$  influx (Figure 6.1).



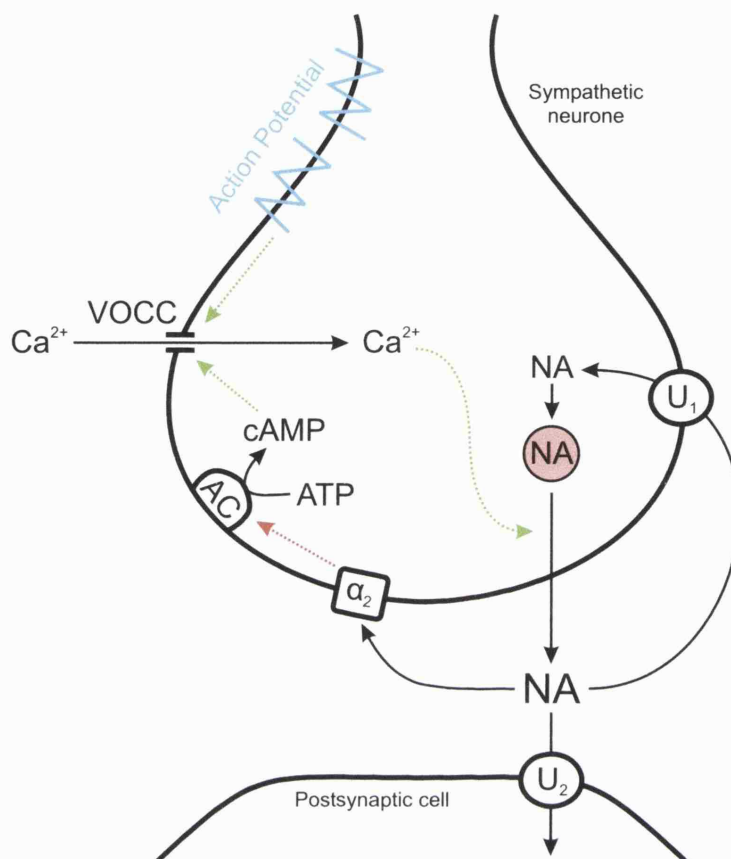


Figure 6.1: Schematic showing the mechanisms involved in the release of noradrenaline from sympathetic nerve terminals and its uptake from the synapse by neighbouring cells. NA = noradrenaline, VOCC = voltage operated calcium channel, U<sub>1</sub> = Uptake<sub>1</sub>, U<sub>2</sub> = Uptake<sub>2</sub>, AC = adenylate cyclase, ATP = adenosine-5-triphosphate, cAMP = cyclic-3,5-adenosine-monophosphate,  $\alpha_2$  =  $\alpha_2$ -adrenoceptor. Red arrows denote inhibition, Green arrows denote activation. Adapted from Rang & Dale, 1991.

Unlike the neurotransmitter acetylcholine, whose actions are terminated by enzymic degradation in the synapse, NA is removed intact from the synapse by two separate uptake mechanisms, one located on neuronal cells (Uptake<sub>1</sub>) and the other on non-neuronal cells (Uptake<sub>2</sub>) (van Euler US, 1972; Fleig HA *et al.*, 1987). These two mechanisms combine to terminate the synaptic actions of NA (Figure 6.1). After reuptake, NA is either recycled back into the secretory vesicles or is broken down by metabolizing enzymes. Uptake<sub>1</sub> has been demonstrated in the nasal mucosa of the pig: enhanced NA vasoconstriction was detected following inhibition of Uptake<sub>1</sub> by desipramine (Lacroix JS *et al.*, 1989). Neuronal uptake has also been demonstrated in rabbit nasal tissue, as uptake of (<sup>3</sup>H) NA was significantly reduced following administration of cocaine or sympathectomy (de la Lande IS *et al.*, 1987).

Uptake<sub>2</sub> protein mRNA has been found in human bronchial smooth muscle cells (Horvath G *et al.*, 2003), and the Uptake<sub>2</sub> inhibitor, normetanephrine (NM), potentiated the actions of isoprenaline in pig bronchi (Foster PS *et al.*, 1983). The relative contribution of Uptake<sub>1</sub> and Uptake<sub>2</sub> to the termination of NA activity depends on the density of sympathetic neurones in the tissue in question. In the lung, Uptake<sub>2</sub> is thought to predominate, due to a relative sparsity of sympathetic neurones (Horvath G *et al.*, 2003). Whereas, in nasal tissue highly innervated by sympathetic terminals, Uptake<sub>1</sub> is thought to predominate (de la Lande IS *et al.*, 1987).

The metabolism of NA is mediated by a complex system of cytosolic enzymes, as shown in Figure 6.2. The two main enzymes, monoamine oxidase and catechol-O-methyl transferase, are generally only found in neuronal and non-neuronal cells, respectively (Kopin IJ, 1972; Fleig HA *et al.*, 1987; Branco D *et al.*, 1992). In the one publication investigating NA metabolism in nasal tissue (rabbit), 3,4-dihydroxyphenylglycol (DHPG) was shown as the main metabolite of NA, with NM predominating after administration of cocaine or sympathectomy (de la Lande IS *et al.*, 1987).

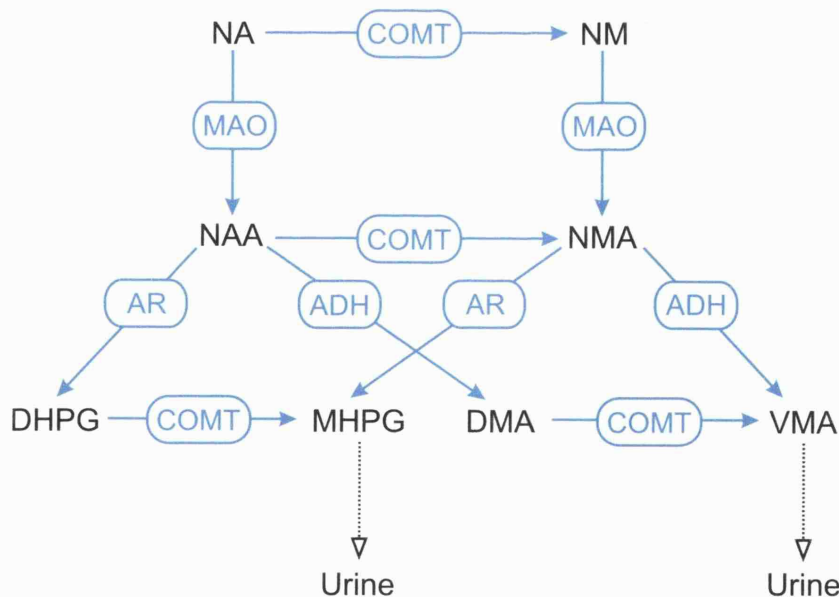


Figure 6.2: The main pathways of noradrenaline metabolism. NA = noradrenaline, NM = normetanephrine, NAA = NA aldehyde, NMA = NM aldehyde, DHPG = 3,4-dihydroxyphenylglycol, MHPG = 3-methoxy, 4-hydroxyphenylglycol, DMA = 3,4-dihydroxymandelic acid, VMA = vanillylmandelic acid. Blue arrows denote conversion of the

substrate by the labeled enzyme. COMT = catechol-O-methyl transferase, MAO = monoamine oxidase, AR = aldehyde reductase, ADH = aldehyde dehydrogenase. Adapted from Rang & Dale, 1991.

The three drugs used in this chapter are lidocaine, clonidine and corynanthine. Lidocaine, the potent local anaesthetic, has a rapid rate of onset and has a half-life of 2 hours (Covino BG, 1987). Clonidine is a selective partial agonist at  $\alpha_2$ -adrenoceptors (Stump DC *et al.*, 1983; Chapleo CB *et al.*, 1989), with no effect at  $\alpha_1$ -adrenoceptors (Tanaka T *et al.*, 1980). Corynanthine is a highly selective  $\alpha_1$ -adrenoceptor antagonist, with a  $\alpha_2/\alpha_1$  selectivity ratio of 0.03 (Doxey JC *et al.*, 1984).

## 6.2 Development of the noradrenaline assay

Although HPLC techniques have been used to measure NA in urine, plasma and cerebrospinal fluid (Larsson M *et al.*, 1987; Esler M, 1993; Willemsen JJ *et al.*, 2001), NA detection in nasal lavages has not been previously reported in the literature. In this section the development of an assay suitable for the detection of NA in nasal lavage is described.

NA is not completely stable. *In vivo* it can be metabolized by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (Trendelenburg U, 1984) and has an *in vivo* half-life of approximately 2 minutes (Grimm M *et al.*, 1980). *In vitro* it decomposes naturally (Hughes IE *et al.*, 1978). NA can be stabilized by cold, dark and acidic conditions (in which it can resist decomposition for many hours). Thus the assay needed to maximize the recovered NA and prevent its degradation.

The HPLC-ECD used for the development of the NA assay had a resolution limit of approximately 0.2nM (10fmol in 50µl) and yielded a standard NA peak at approximately 5.5 to 6 minutes. As the following experiments were performed on 1 or 2 individuals; no statistical analyses have been performed. As NA is stabilized in cool and dark conditions, every effort was made to keep standards and samples in dark refrigeration for as long as possible.

### 6.2.1 Initial lavage assay

Three 5ml nasal lavages were performed, as described in Chapter 2.3.1; the third was collected. A drop of 10% perchloric acid (HClO<sub>4</sub>) was added to acidify the solution. The sample was vortexed and then centrifuged (1400 x g) for 20 minutes. The supernatant was analysed (50µl injection volume). (n=2)

The lavage traces were very smooth, except for the non-retained solvent front, suggesting the samples did not contain many oxidizable molecules after separation by the columns. Sample (N) yielded a peak at 5.5 minutes, which was calculated to be a peak the equivalent of ~0.6nM NA, as shown in Figure 6.3. Sample (T) did not yield a peak, data not shown.

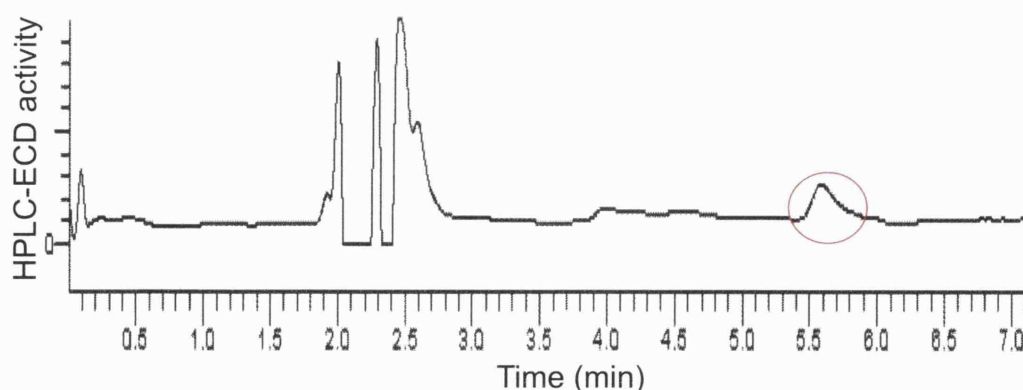


Figure 6.3: HPLC-ECD chromatogram showing the peaks of oxidative activity of the lavage sample (N) after it has been separated by the HPLC. The red circle encloses the peak which corresponds to the concentration of NA in lavage sample (N). After comparing this peak with the peak derived from a known NA standard, the concentration of NA was calculated to be  $\sim 0.6$  nM. The peaks at 2 minutes correspond to the solvent front.

### 6.2.2 Spiked lavage assay

Three 5ml nasal lavages were performed; the third was collected. A drop of 10%  $\text{HClO}_4$  was added. The sample was vortexed and then centrifuged ( $1400 \times g$ ) for 20 minutes. The supernatant was split, with one aliquot spiked with NA. The samples were then analysed (50  $\mu\text{l}$  injection volume). ( $n=1$ )

The combination of NA spike and lavage sample yield only one peak with the same peak time as the NA standard, suggesting the lavage contained NA. However, qualitatively, the combination peak was not as large as was expected:

Known NA standard: 1.2 nM

Lavage sample: 0.705 nM

A combined solution of equal volumes of NA standard and lavage sample should have yielded an equivalent peak of 0.953 nM. However, the peak was only the equivalent of 0.812 nM NA; a deficit of 0.141 nM NA (approximately 15%). This reduction of NA could be explained by the presence of metabolizing enzymes in the lavage.

### 6.2.3 Additional centrifugation

The aim of this assay was to remove further any metabolizing enzymes from the sample. The method was a repeat of the method used in 6.2.1, except that the supernatant was centrifuged again (20 minutes at 1400 x g) and the subsequent supernatant was analysed (50µl injection volume). (n=1)

No peaks were obtained, data not shown.

### 6.2.4 Additional vortex following second centrifugation

The aim of this assay was to prevent the build up of discrete layers of NA in the sample after the repeated centrifugation. The method was a repeat of the method used in 6.2.3, except the supernatant was vortexed following the second centrifugation. (n=1)

No peaks were obtained, data not shown.

### 6.2.5 Increased acid, kept on ice.

The aim of this assay was to stabilize further the NA in the lavage samples by lowering the pH and temperature. Three 5ml nasal lavages were performed, and the third was collected. 1ml 10% HClO<sub>4</sub> was added to 4.5ml of sample and was then vortexed briefly. The sample was centrifuged (1400 x g) at 4°C for 5 minutes. Supernatant was vortexed and then analysed three times (50µl injection volume). (n=2)

Sample (N) yielded a mean peak equivalent of 0.88nM NA. Sample (T) yielded a small peak, at the limits of resolution, and so it was immeasurable.

### 6.2.6 Lavage sample measured over time; first lavage analysed; increased acid.

The aim of this experiment was to assess the effect of time on the measured concentration of NA in a nasal lavage sample under cold and acidic conditions. No

pre-washes were performed so as to maximize the NA levels. Only one 5ml lavage was performed (no pre-washes). 1ml 0.5M  $\text{HClO}_4$  was added to 4ml of sample and was then vortexed briefly. The sample was centrifuged ( $1400 \times g$ ) at  $4^\circ\text{C}$  for 5 minutes. Supernatant was vortexed and then analysed (50 $\mu\text{l}$  injection volume). (n=1)

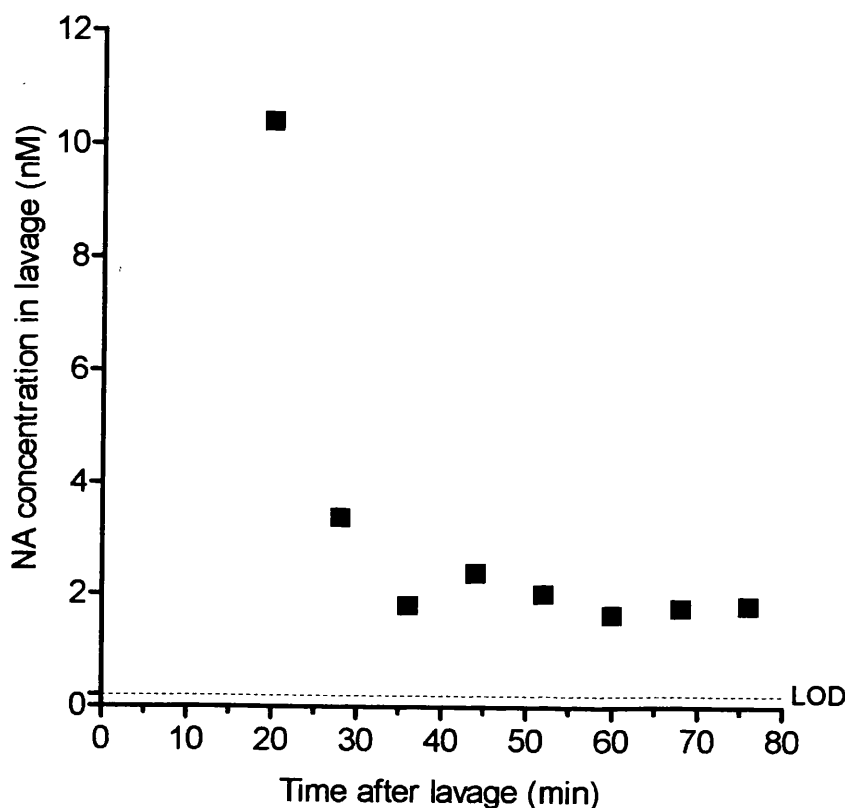


Figure 6.4: The effect of time on the noradrenaline [NA] concentration in a single lavage sample, as measured by HPLC-ECD and as compared to known NA standards. After lavage was collected and processed, the same sample was injected into the HPLC every 8 minutes. The peak time of the sample corresponded to that derived from a NA standard. The sample peaks were then compared to the NA standard peaks and the NA concentration was calculated for each point. Only one injection/analysis at each time point was performed. Limit of detection [LOD] was  $\sim 0.2\text{nM}$ .

The lavage NA concentration dropped rapidly by 60% from 20 to 36 minutes after the lavage was taken (Figure 6.4). However, the level of NA then appeared to stabilize at approximately  $1.5\text{nM}$  for the duration of the experiment. This level is above the resolution of the detection ( $0.2\text{nM}$ ). NA alone in these conditions ( $\sim\text{pH } 4$ ,  $4^\circ\text{C}$ , in the dark) should not decay this rapidly. NA decay was measured previously

(4°C, pH 5.7) and showed that 2.5nM NA decayed exponentially ( $[NA] = 116.6 \cdot e^{(-0.0038 \cdot \text{time})}$ ) to 2.2nM in 15 minutes and 1.8nM by 60 minutes (S Geranton, personal communication). The curve in Figure 6.4 does not suggest simple NA decay, as the NA level would continue to dip towards the limits of resolution. Similarly, NA metabolism is unlikely to be the cause as the recorded NA level would again drop towards the limits of resolution. The cool and acidic conditions would be expected to prevent the activity of NA-metabolizing enzymes such as MAO and COMT (pKa of 6.2 and 8.4, respectively). HClO<sub>4</sub> has been used extensively to prevent enzymic degradation of NA in plasma samples (Falconer AD *et al.*, 1982) and the tell-tale white protein precipitate usually observed in plasma samples after the application of acid was not detected in these lavages.

There remains the possibility that the first two data points shown in Figure 6.4 are anomalous results. Whether this is due to catecholamine contamination in the HPLC or an error in the electrochemical detection is not clear.

### 6.2.7 Spiked lavage compared with NA standard

The aim of this experiment was to assess the effect of the assay process on the concentration of NA in a known standard, a lavage, and a lavage spiked with a known standard. Three separate solutions were made up for analysis:

- A) Known spike in saline: 2.4nM NA in 5ml sterile saline. The solution was briefly vortexed.
- B) Lavage: One 5ml lavage was performed. 4 ml of sample were taken and added to 1ml of 0.5M HClO<sub>4</sub> and vortexed briefly.
- C) Lavage plus known spike: 3.96ml of the same lavage sample was added to 1ml of 0.5M HClO<sub>4</sub> plus 0.04ml of 0.24μM NA. The solution was briefly vortexed.

The three solutions were centrifuged (1400 x g) in a pre-cooled (4°C) centrifuge for 5 minutes. The supernatants was removed and vortexed. The samples were then analyzed (50μl injection volume) and compared to 2.4nM NA dissolved in distilled water (not having been subjected to the refinement process). (n=1)



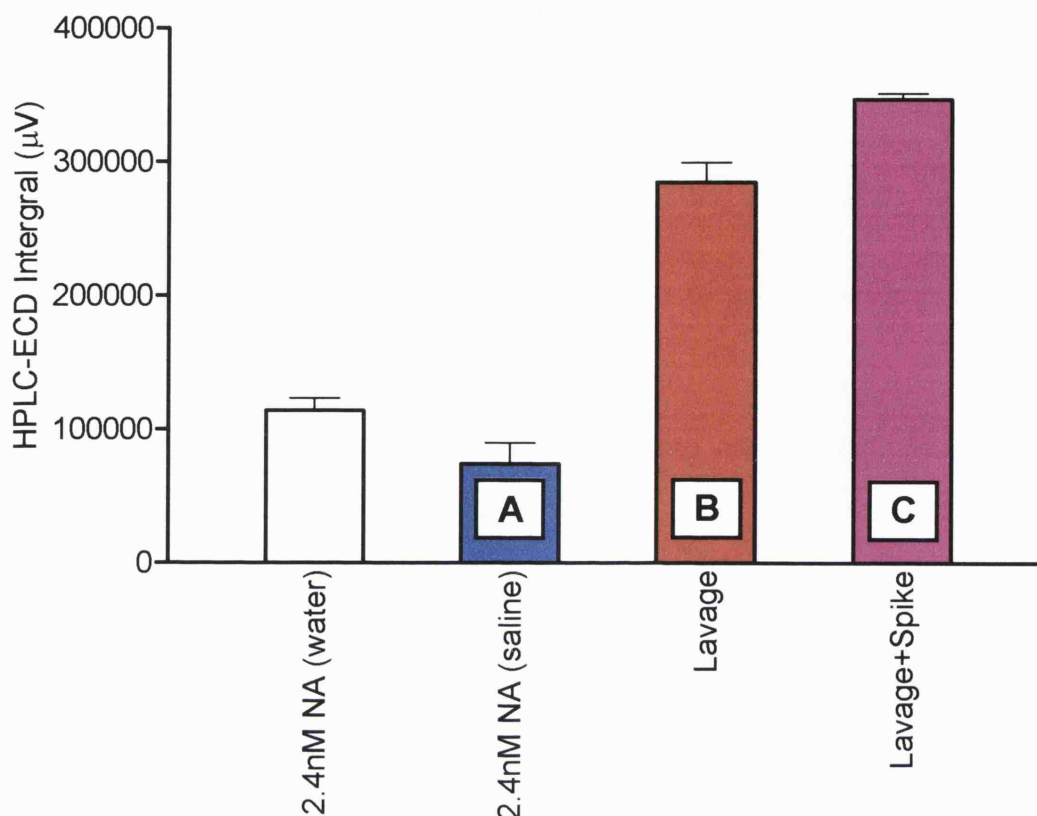


Figure 6.5: Levels of oxidative activity following analysis of noradrenaline [NA] standards and a single lavage sample, as measured by HPLC-ECD (50μl injection volume). Each column represents the mean of 3 peak integral measurements of the same solution, with the vertical bars representing the s.e.mean. After a lavage was collected the sample was split into two, with one solution given a spike of NA, the equivalent of the NA standard. Both lavage solutions, the lavage alone [B] and the spiked lavage [C], together with the NA standard in saline [A] were then processed before analysis.

The lavage [B] peak time was identical to the NA standards, and the lavage plus spike [C] yielded only one peak at the time that NA standards produced a peak, indicating that the lavage did, in fact, contain NA.

However, the HPLC-ECD integral for the 2.4nM NA saline spike [A] is clearly far lower than the integral for the 2.4nM NA in water control (Figure 6.5). A difference of 35% is not expected between two solutions with the same NA concentration. This suggests that the refinement process is, to a significant degree, reducing the levels of NA in the samples. This reduction of 35% of NA should occur proportionally to each sample. If the integrals (measure of NA concentration) for [B] and [C] are

corrected for their dilution by the added acid,  $[A] + [B]$  should equal  $[C]$ . Using the corrected integrals,  $[A] + [B] = 0.982[C]$ . Therefore, the data suggests that the amount of NA lost, apparently due to the refinement process, is proportional to the NA concentration in the sample, indicating that relative changes in NA levels are consistent. However, the data could also be explained by incorrectly high measurements made of the initial NA in water standard.

### 6.2.8 Saline analysis

The aim of this experiment was to investigate the effect of saline on HPLC-ECD integrals. Saline was repeatedly injected into the HPLC for analysis (50 $\mu$ l injection volume).

The results were inconsistent; although most chromatograms showed no effect (data not shown), one test yielded a measurable peak and another test yielded a *negative* peak.

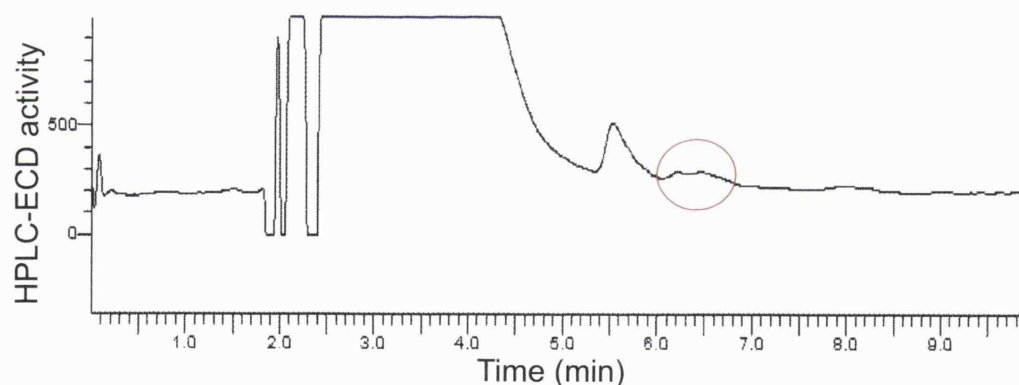


Figure 6.6: HPLC-ECD chromatogram showing the peaks of oxidative activity following injection of saline into the HPLC. The red circle encloses the peak which corresponds to the time at which a NA peak would appear.

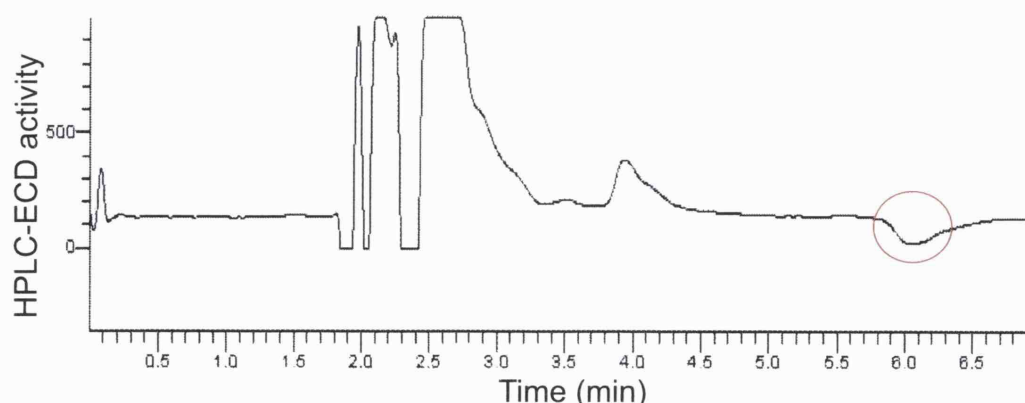


Figure 6.7: HPLC-ECD chromatogram showing the peaks of oxidative activity following injection of saline into the HPLC. The red circle encloses the peak which corresponds to the time at which a NA peak would appear.

The data suggest that saline has little or no effect on HPLC-ECD peaks at 5.5 to 6 minutes, although some recordings, as shown in Figures 6.6 and 6.7, indicate that the HPLC-ECD was affected by a contamination problem. No effect of saline on HPLC-ECD peaks has been previously reported.

### 6.2.9 Overview

In order to investigate the levels of NA in nasal lavages, a suitable assay had to be developed. Unfortunately, the development of this assay was hindered by the apparent unpredictable absence of the substance that the assay was being developed for. Approximately 45% of lavages either yielded no NA peaks or the peaks were lower than the limit of detection. These recorded absences of NA could have been examples of unsuitable assay conditions. However, the initial assay conditions would be expected to stabilize any NA and precipitate any NA-binding proteins. Nevertheless, as the assay developed (more acid, colder and reduced centrifugation), measurable NA levels became more frequent. The NA concentrations measured in the various assays and experiments for the two subjects used (Subject T and Subject N) are shown in Figure 6.8.

Each data point in Figure 6.8 represents a peak in an analysed lavage which has the same peak time as the NA standard used that day. These numerous measurable observations, together with the single peaks derived from spiked

lavages, suggest that NA is often present in nasal lavages, albeit at levels close to the HPLC-ECD limit of detection.

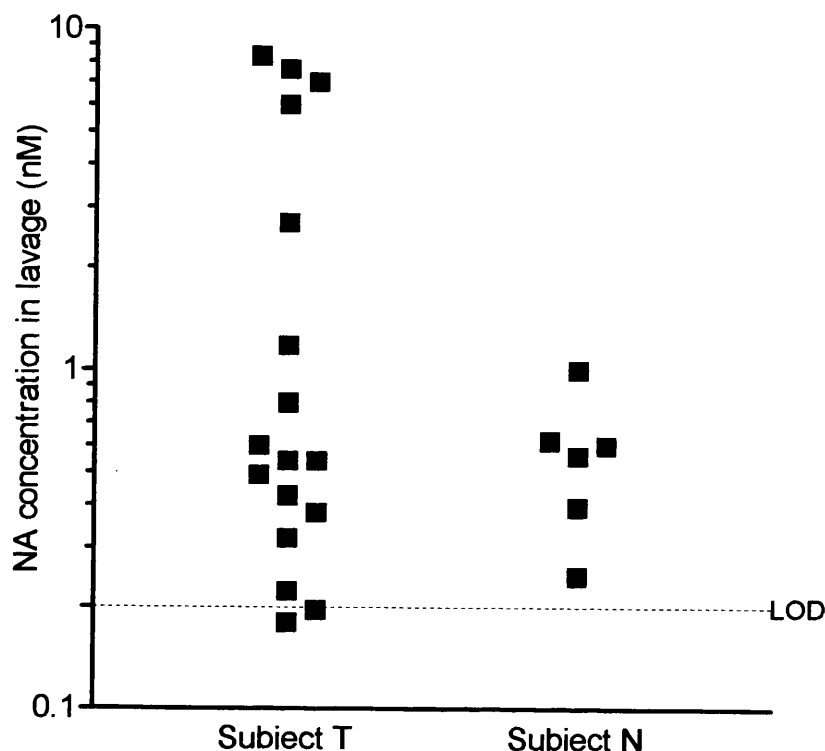


Figure 6.8: Overall spread of lavage NA concentrations measured throughout the development of the NA lavage assay by HPLC-ECD. Data were converted from peak area into NA concentration after comparing with known NA standards. Each data point represents the mean NA concentration calculated from a given lavage from subjects T and N. Limit of detection [LOD] was ~0.2nM.

However, it is clear that the measurement of NA using this HPLC-ECD system was not reliably quantitative. In particular, as shown in Figures 6.4 and 6.5, the measured levels of NA were widely different to what was expected. The acidic, cold and dark conditions should have stabilized the NA for days (Falconer AD *et al.*, 1982), but instead measured NA levels fell by 80% within 16 minutes in Chapter 6.2.6 (Figure 6.4) and a NA standard fell by 35% in Chapter 6.2.7 (Figure 6.5). It seems unlikely that the actual NA concentration was decaying at this rate. In conclusion, NA was probably present in nasal lavages but this in-house HPLC-ECD was not capable of more exact measurements.

### 6.3 Catecholamine screening of nasal lavages

The development of the NA assay using the in-house HPLC-ECD demonstrated that NA stability could be increased using acidic, cold and dark conditions, as well as limiting the mechanical stress (vortex and centrifugation) the samples were subjected to. In addition, as the detected NA concentrations were close to the limits of detection, the following study utilized 2.5ml lavages as opposed to 5ml lavages, as well as concentrating down the samples using an alumina extraction kit.

The nasal lavage samples were screened for a range of catecholamines in order to assess not just the levels of sympathetic mediators, but their metabolites as well.

#### 6.3.1 Protocol

Three 2.5ml lavages were performed, as described in Chapter 2.3.1, 10 minutes apart. The first (initial) lavage and third (final) lavage were collected in separate pre-cooled (4°C) centrifuge tubes. 0.4ml of 0.5M HClO<sub>4</sub> was added to each lavage. The solution was briefly vortexed and centrifuged (1400 x g) in a pre-cooled (4°C) centrifuge for 5 minutes. 1.5ml of supernatant was then used for the extraction of catecholamines using an alumina column, as described in Chapter 2.3.2. The extracted catecholamines, concentrated into 200µl eluting solution, were stored in dry ice and transported to Pfizer, Sandwich, UK for analysis. Four subjects took part in the study.

In addition, a negative control and a positive NA control (0.907nM after addition of acid) were processed using the same methodology as the lavages and sent to Pfizer, Sandwich, UK for analysis.

#### 6.3.2 Results

The chromatogram for the eluting buffer (negative control) showed a smooth trace, with the exception of the non-retained solvent front, suggesting the eluting buffer did not contain many oxidizable molecules (Figure 6.9). The chromatogram for the NA spike (positive control) had a very large peak at 4 minutes, corresponding to a large amount of NA in the injected sample (Figure 6.10). Although there was another

minor spike at 7 minutes, this did not correspond to any known oxidizable catecholamine. Aside from the NA peak, the NA spiked sample did not produce any other catecholamine peaks, suggesting that the extraction process does not breakdown NA into its metabolites such as DHPG or MHPG.

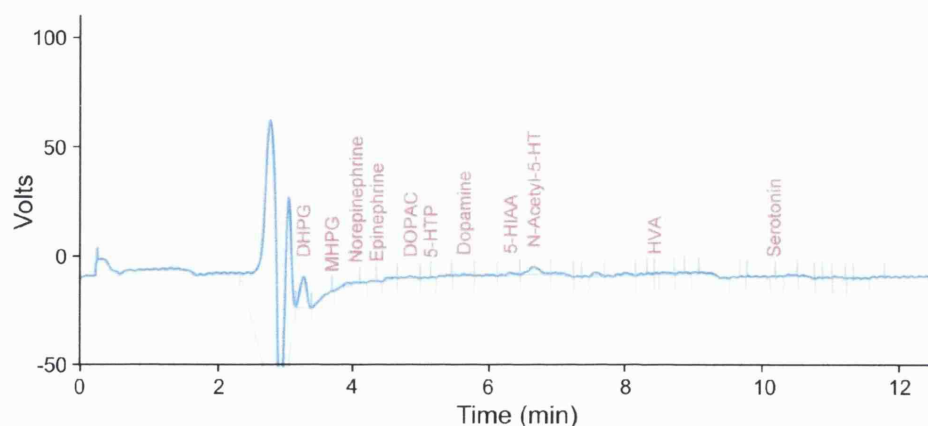


Figure 6.9: HPLC-ECD chromatogram showing the peaks of oxidative activity following injection of eluting buffer into the HPLC.

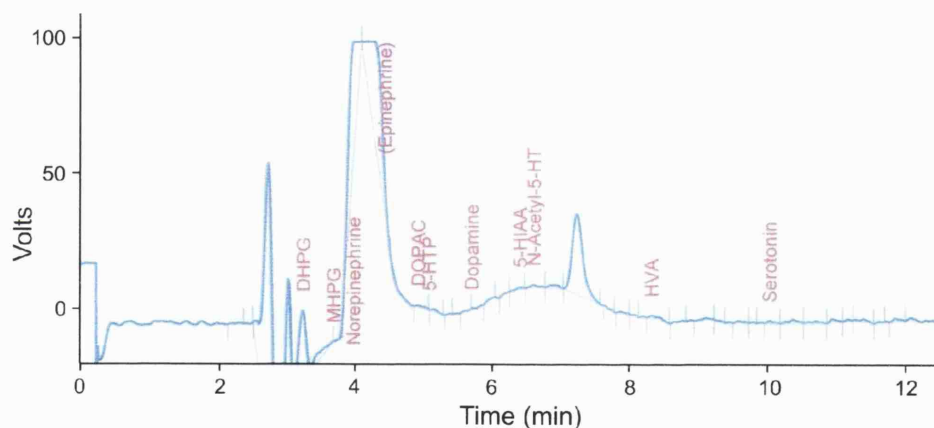


Figure 6.10: HPLC-ECD chromatogram showing the peaks of oxidative activity following injection of NA (norepinephrine) standard into the HPLC.

The chromatograms (Subjects A to D) for the initial (Figure 6.11) and final (Figure 6.12) lavages all show large peaks at approximately 3 minutes, indicating high levels of DHPG. In addition, most chromatograms show a minor peak for adrenaline (epinephrine) at 4.5 minutes. There are no significant peaks for NA, MHPG, dopamine (or its metabolites) and serotonin (or its metabolites) in the lavage analyses. The mean levels of DHPG, NA and adrenaline in the nasal lavages are

shown in Figures 6.13 and 6.14 (catecholamine levels calculated from area under the peaks or from the height of the peaks, respectively). There is little difference between the two graphs, showing that both techniques for evaluating catecholamine levels produce similar data. There is no significant difference in DHPG levels between initial and final lavages ( $p > 0.05$ , Wilcoxon sign-rank test).



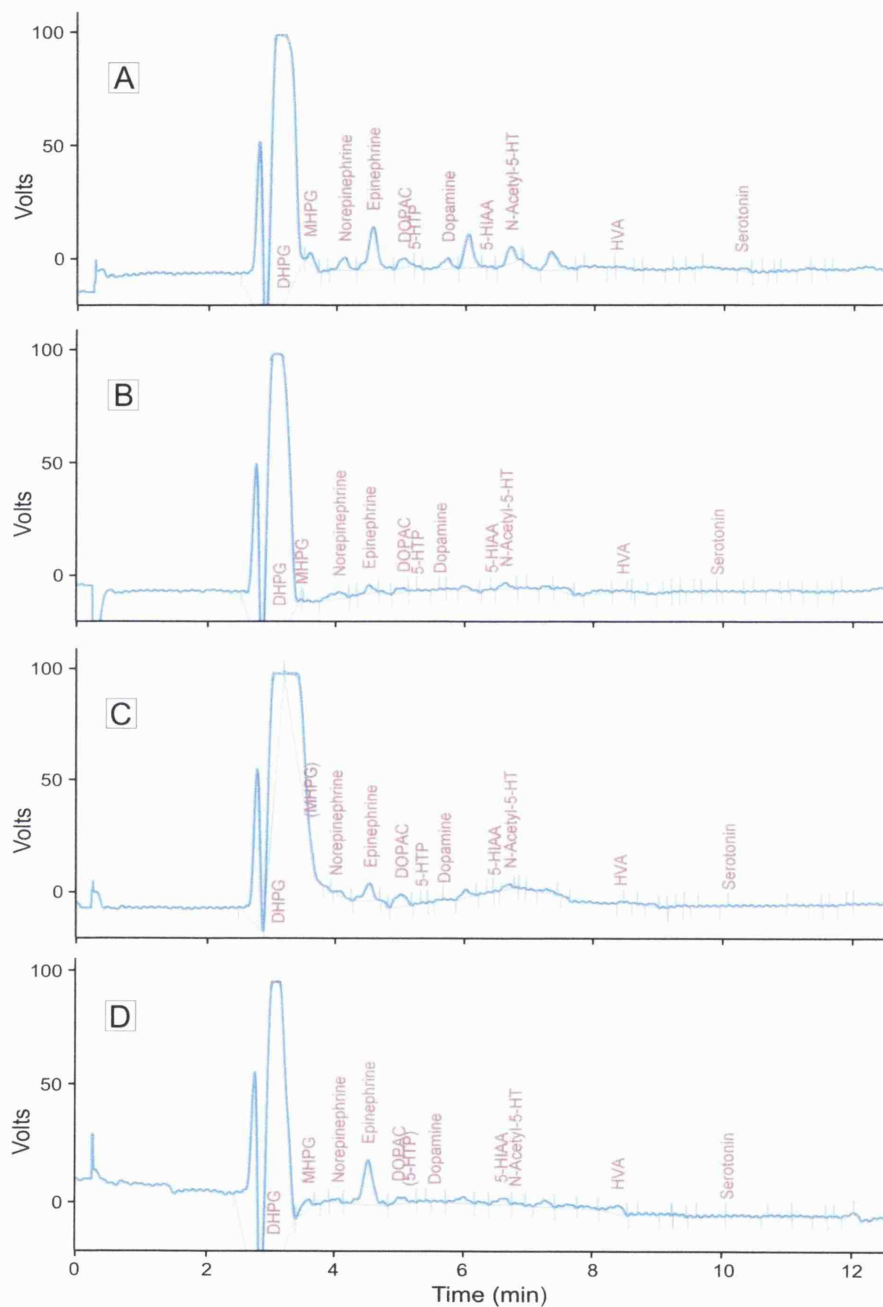


Figure 6.11: HPLC-ECD chromatograms showing analysis of initial nasal lavages from 4 separate subjects (A to D). For each subject, both nostrils were lavaged using saline, 2.5ml. The catecholamines present in the recovered lavage samples were extracted and injected (30 $\mu$ l) into the Pfizer HPLC-ECD. DHPG = 3,4-dihydroxyphenylglycol.



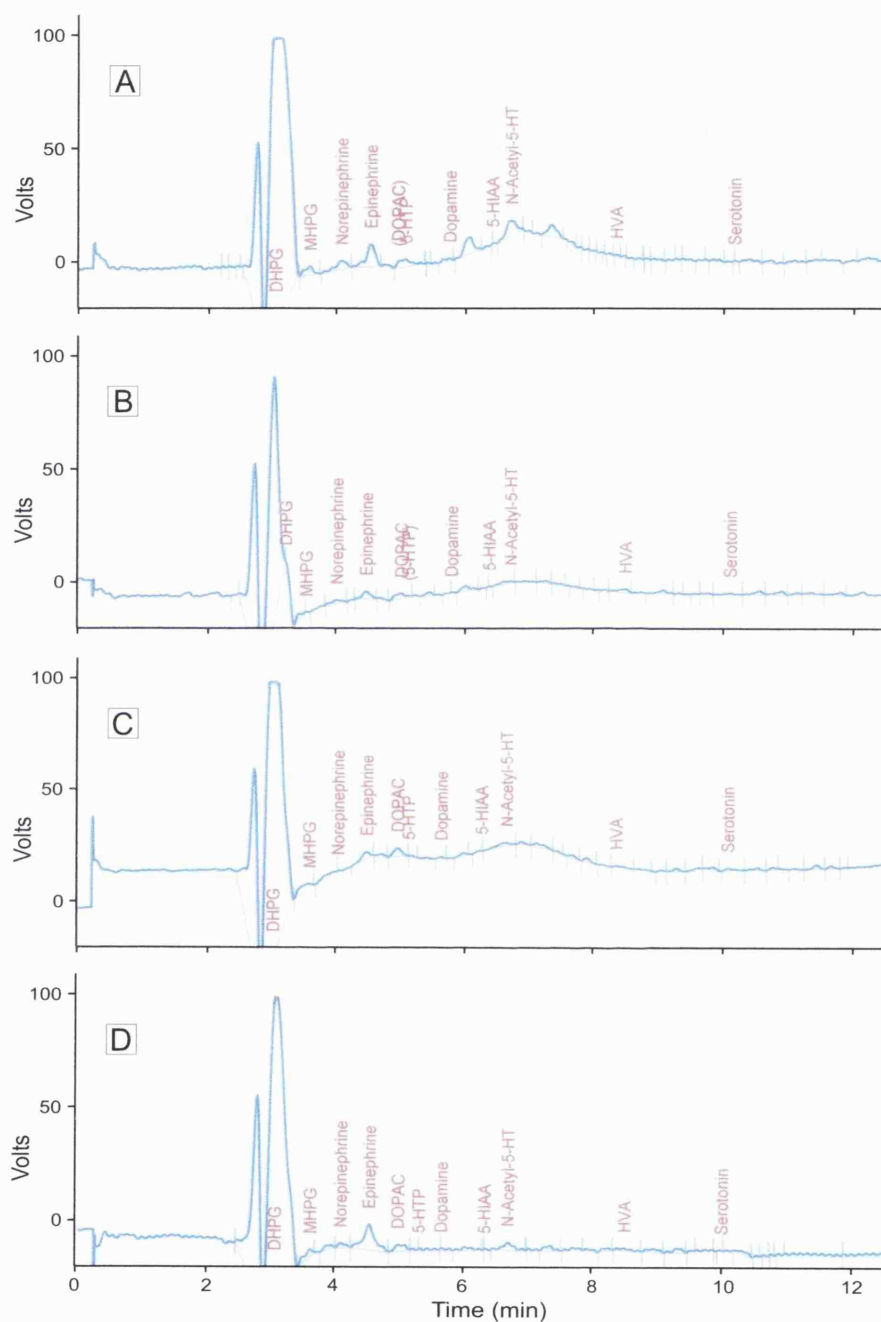


Figure 6.12: HPLC-ECD chromatograms showing analysis of final nasal lavages from 4 separate subjects (A to D). For each subject, both nostrils were lavaged three times using saline, 2.5ml. The catecholamines present in the samples recovered from the third lavage were extracted and injected (30 $\mu$ l) into the Pfizer HPLC-ECD. DHPG = 3,4-dihydroxyphenylglycol.

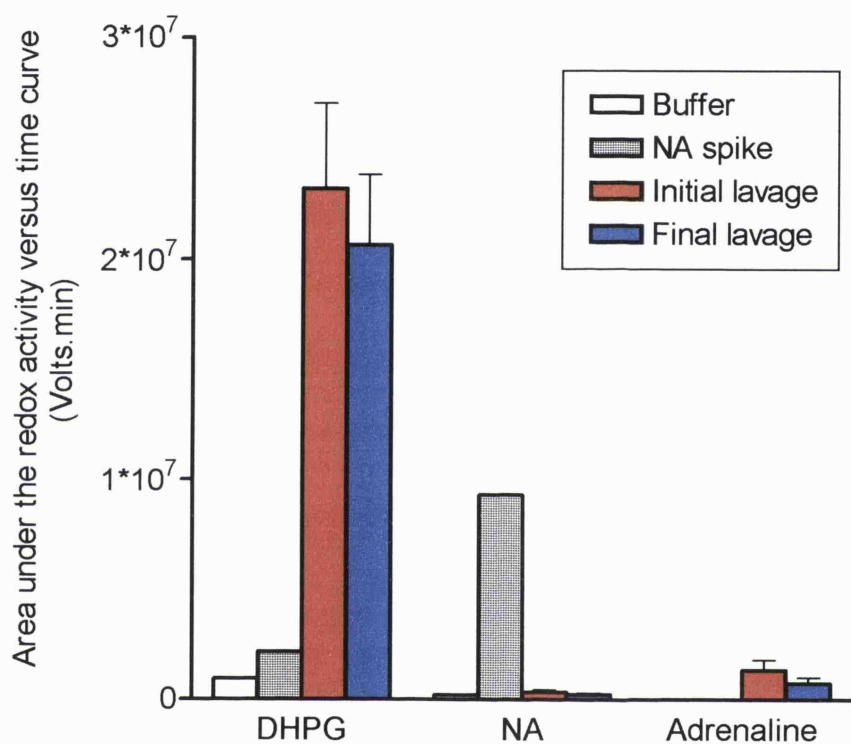


Figure 6.13: The levels of catecholamines in nasal lavages as measured by area under the relevant peaks recorded by HPLC-ECD. Data for initial [red] and final [blue] lavages are means from 4 subjects, vertical bars represent s.e.mean. Data represent the area under the redox activity versus time curve.

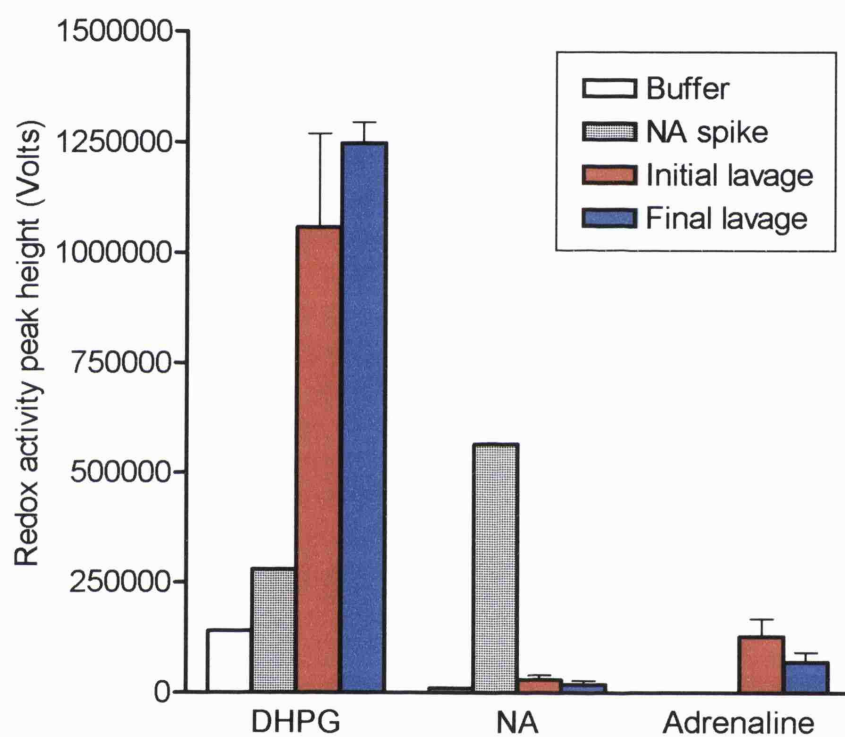


Figure 6.14: The levels of catecholamines in nasal lavages as measured by the height of the relevant peaks recorded by HPLC-ECD. Data for initial [red] and final [blue] lavages are means from 4 subjects, vertical bars represent s.e.mean. Data represent the peak height.

## 6.4 Experimental protocol for functional experiments

In the following double-blind experiments, healthy non-atopic human volunteers were used as subjects.

### 6.4.1 The effect of topical local anaesthetic, lidocaine, on nasal patency

The aim of this experiment was to investigate the effect of neuronal blockade on nasal patency, as measured by acoustic rhinometry. Subjects received, by 100µl aerosol into each nostril, either saline or lidocaine, 4mg. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry and visual analog scores (VAS) for nasal anaesthesia were completed by the subjects. 8 subjects were included in this study. The dose and time-course used were based upon pilot studies, previous experiments (Turner PJ, 1999) and the published potency of the local anaesthetic, lidocaine (Covino BG, 1987). The protocol is summarized in Figure 6.15.

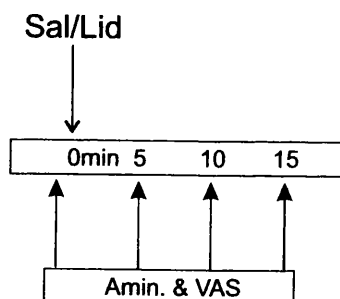


Figure 6.15: Protocol for the effect of lidocaine on nasal patency. Sal = saline, Lid = lidocaine.

### 6.4.2 The effect of topical $\alpha_2$ -adrenoceptor agonist, clonidine, on nasal patency

The aim of this experiment was to assess whether the activation of presynaptic autoreceptors by clonidine would reduce the release of endogenous NA and thus cause nasal blockage, as measured by acoustic rhinometry. Subjects received, by 100µl aerosol into each nostril, either saline or clonidine, 100µg. Each subject

received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area ( $A_{min.}$ ) of the nasal airways was measured using acoustic rhinometry. 7 subjects were included in this study. The dose and time-course used were based upon nasal bioavailability in rats (Babhair SA *et al.*, 1990), the doses used in the treatment of hypertension and the potency of topical clonidine on the eye (Abrams DA *et al.*, 1987). The protocol is summarized in Figure 6.16.

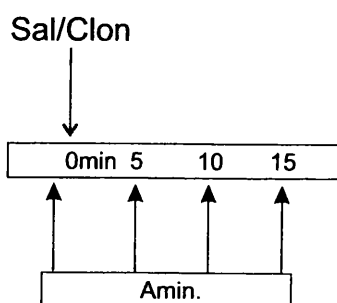


Figure 6.16: Protocol for the effect of clonidine on nasal patency. Sal = saline, Clon = clonidine.

#### 6.4.3 The effect of topical $\alpha_1$ -adrenoceptor antagonist, corynanthine, on nasal patency

The aim of this experiment was to assess whether the inhibition of  $\alpha_1$ -adrenoceptors by corynanthine would reduce the basal contraction of nasal blood vessels by endogenous NA and thus cause nasal blockage, as measured by acoustic rhinometry. Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline or corynanthine, 2mg. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area ( $A_{min.}$ ) of the nasal airways was measured using acoustic rhinometry. 11 subjects were included in this study. The dose and time-course used were based upon the published topical dose used in a study investigating the effect of corynanthine on intraocular pressure in man (Serle JB *et al.*, 1985). The protocol is summarized in Figure 6.17.

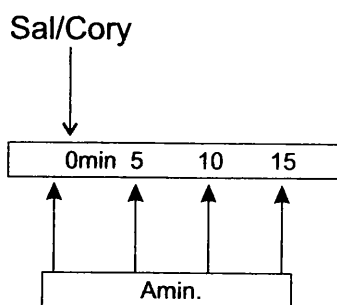


Figure 6.17: Protocol for the effect of corynanthine on nasal patency. Sal = saline, Cory = corynanthine.

#### 6.4.4 Data analysis

Nasal patency was measured using acoustic rhinometry, as described in Chapter 2. Amin. was recorded immediately prior to and 5, 10 and 15 minutes after nasal challenge. Amin. values were normalised to the Amin. value recorded prior to nasal challenge. For each challenge, a normalised Amin. versus time curve was plotted, and the area under the curve (AUC) calculated. Subjective symptom scores of nasal anaesthesia were assessed using visual analog scores (VAS), as described in Chapter 2. Post-challenge VAS were corrected for any recorded pre-challenge value by subtracting the pre-challenge value from the recordings made following nasal challenge. For each challenge, a corrected VAS versus time curve was plotted, and the area under the curve calculated. Thus the presented VAS data represents only the total change in symptom scores over a 15 minute period following challenge. The rhinometry and VAS data are presented as mean values, together with s.e.mean. The appropriate non-parametric statistical test is given with each data set.  $p < 0.05$  is taken as significant.

## **6.5 Results for functional experiments**

### **6.5.1 The effect of topical local anaesthetic, lidocaine, on nasal patency**

Application, by aerosol into each nostril, of lidocaine, 4mg, did not caused any significant change in AUC (area under the normalised Amin. versus time curve) as compared to saline ( $p>0.05$ , Wilcoxon sign-rank test), corresponding to no change in nasal patency measured over the 15 minutes after nasal challenge, as shown in Figure 6.18.

Application, by aerosol into each nostril, of lidocaine, 4mg, caused a significant increase in visual analog scores for nasal anaesthesia as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal anaesthesia measured over the 15 minutes after nasal challenge, as shown in Figure 6.19.

### **6.5.2 The effect of topical $\alpha_2$ -adrenoceptor agonist, clonidine, on nasal patency**

Application, by aerosol into each nostril, of clonidine, 100 $\mu$ g, caused a significant increase in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal patency measured over the 15 minutes after nasal challenge, as shown in Figure 6.20.

### **6.5.3 The effect of topical $\alpha_1$ -adrenoceptor antagonist, corynanthine, on nasal patency**

Application, by aerosol into each nostril, of corynanthine, 2mg, caused a significant decrease in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge, as shown in Figure 6.21.

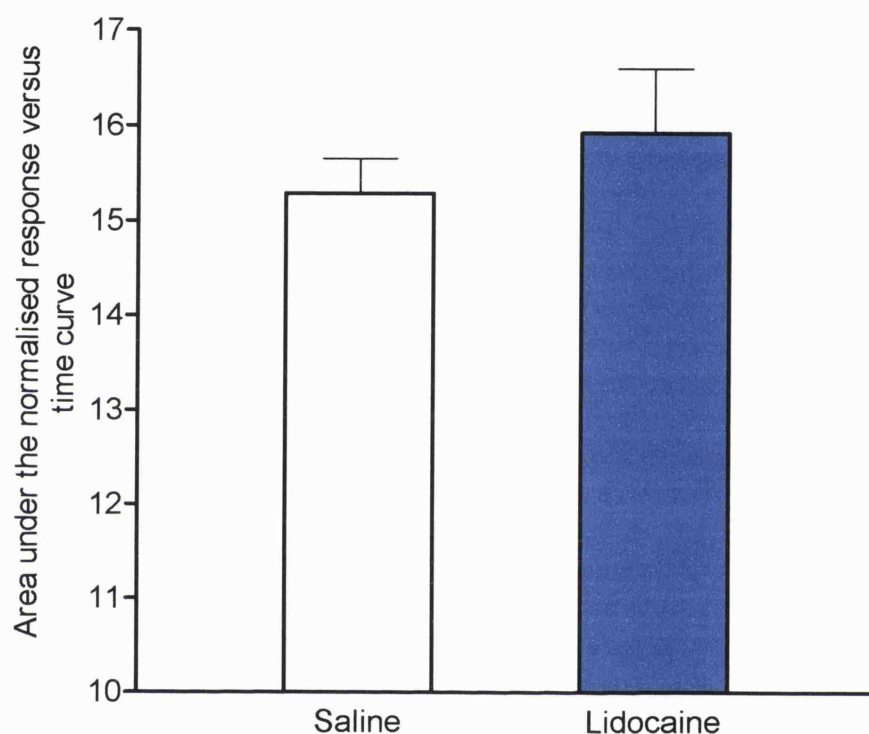


Figure 6.18: The effect of lidocaine on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of lidocaine, 4mg, as an aerosol, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of  $0.682\text{cm}^2$ , s.e.mean of  $0.052\text{cm}^2$ ). Data are means from 8 people. Vertical bars represent s.e.mean.



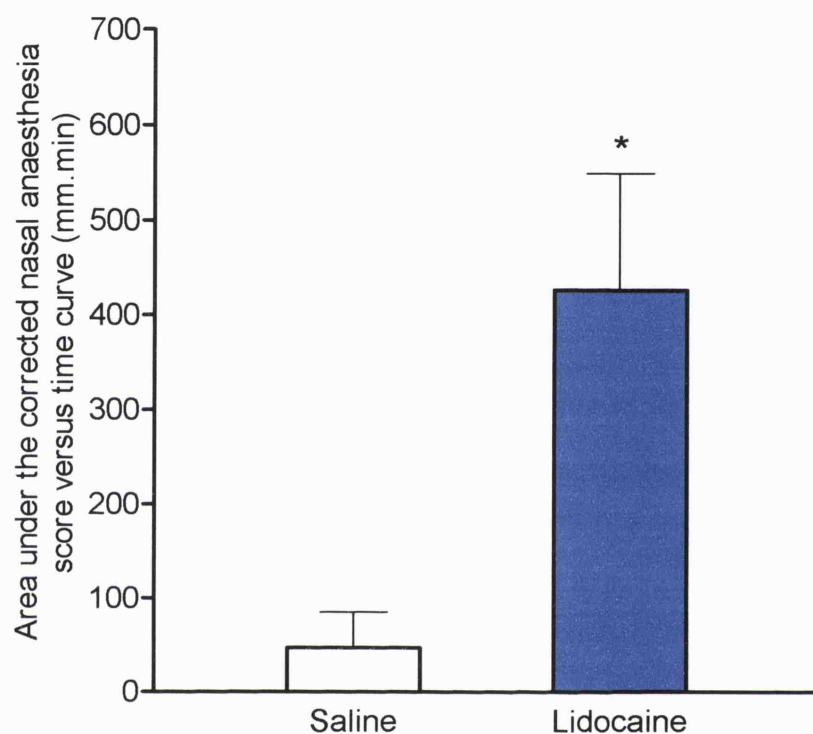


Figure 6.19: The effect of lidocaine on the area under the corrected visual analog score for nasal anaesthesia measured over a period of 15 minutes following the administration of lidocaine, 4mg, as an aerosol into each nostril. Visual analog scores for nasal anaesthesia were measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Visual analog scores were corrected for any measured pre-challenge value by subtracting the pre-challenge response (mm) from the recorded post-challenge responses (mm). The corrected VAS were then plotted against time and the area under the curve calculated. Data are means from 8 people. Vertical bars represent s.e.mean. \* Significant increase in nasal anaesthesia as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test).

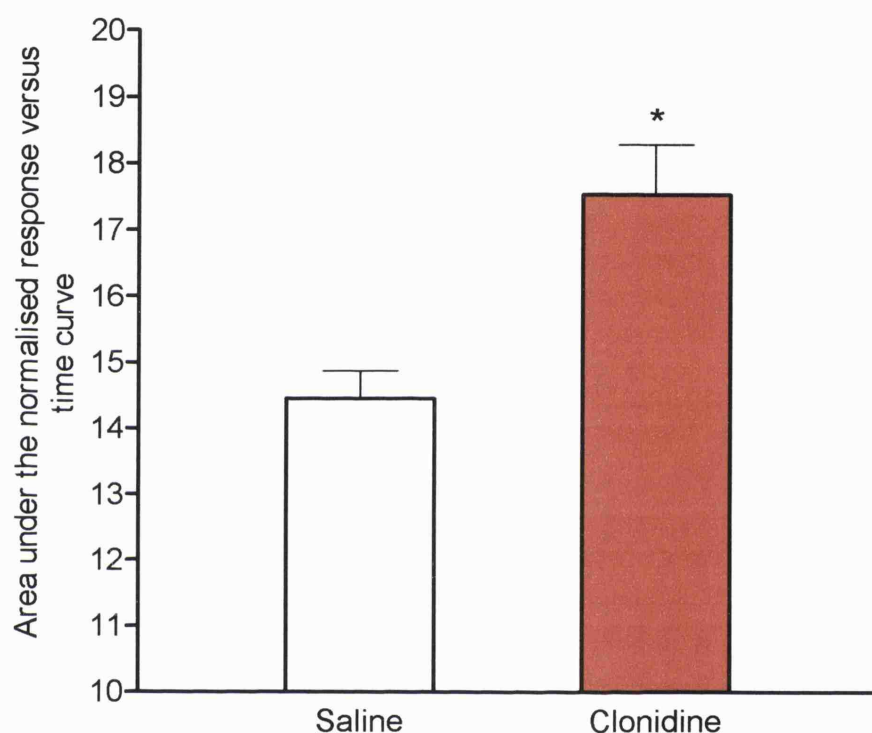


Figure 6.20: The effect of clonidine on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of clonidine, 100µg, as an aerosol, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.660cm<sup>2</sup>, s.e.mean of 0.062cm<sup>2</sup>). Data are means from 7 people. Vertical bars represent s.e.mean. \* Significant increase in nasal patency as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

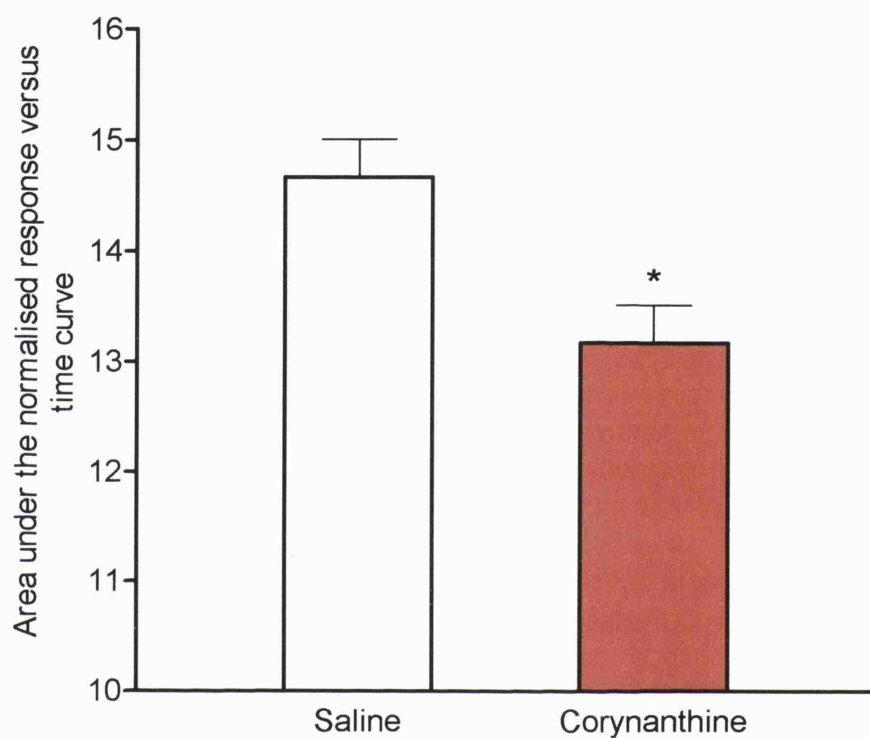


Figure 6.21: The effect of corynanthine on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of corynanthine, 2mg, as an aerosol, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of  $0.710\text{cm}^2$ , s.e.mean of  $0.050\text{cm}^2$ ). Data are means from 11 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

## 6.6 Discussion

Although there is substantial circumstantial evidence to suggest basal sympathetic activity in human nasal mucosa, there is no direct proof in the literature of its control over nasal patency.

The development of the nasal lavage NA assay produced some unexpected results. In general, it was not always clear if NA was present (at detectable levels) in nasal lavages, or if the NA was being broken down, or if the recorded HPLC-ECD analysis was hindered by contamination. NA can degrade naturally or be metabolized by MAO and COMT (Hughes IE *et al.*, 1978; Trendelenburg U, 1984). But, as the assay developed, the acidic, cold and dark conditions used should have been completely capable of stabilizing any NA in the lavages (Falconer AD *et al.*, 1982). Therefore it seems strange that the measured levels of NA underwent such dramatic changes over time (Figure 6.4). The spiked assays were particularly interesting as they showed that there was an oxidizable substance present in the nasal lavage which came off the separation columns at exactly the same time as the added NA. However, analysis of the combined peaks in these spiked NA studies only produce quantitatively reproducible results when the known spike was prepared using the same process as the lavage, as shown in Figure 6.5. When 'processed' NA samples (either known spikes or lavages) were quantitatively compared to 'unprocessed' spikes dissolved in water, the 'processed' peaks were smaller than expected, indicating a negative effect of the refinement process on NA levels.

The data from the NA assays using the in-house HPLC-ECD demonstrated that NA was sometimes present in human nasal lavages, but the levels were very close to the limit of detection for the equipment. NA has previously not been measured in nasal lavages, but NA has been demonstrated in nasal biopsies from pigs, guinea-pigs, rabbits and dogs (Arihood SA *et al.*, 1976; Kubo N *et al.*, 1989a; Stjærne P, 1991).

The analysis of nasal lavages using the Pfizer HPLC-ECD clearly showed high levels of DHPG, very low levels of adrenaline and virtually no measurable NA or MHPG. In addition, there was no evidence of dopamine or serotonin. DHPG is a

lipophilic metabolite of both NA and adrenaline and is an indicator of regional sympathetic activity (Eisenhofer G *et al.*, 1992). Both catecholamines can be broken down by MAO into NA aldehyde which is immediately reduced by aldehyde reductase into DHPG (Kopin IJ, 1972). It is not clear whether the DHPG measured in the nasal lavage is a metabolite of neuronally-released NA or of circulating adrenaline. However, MAO is mainly found in neuronal cells (Branco D *et al.*, 1992) and the majority of adrenaline is initially metabolized by the extraneuronal COMT (Kopin IJ, 1972), therefore it seems unlikely that adrenaline is solely responsible for the DHPG peaks. It would be interesting to measure the levels of COMT metabolites of NA and adrenaline, such as normetanephrine and metanephrine, in nasal lavages. If NA is responsible for the DHPG peaks, it is surprising that no NA could be detected in the lavages. However, it is possible that due to the density of sympathetic neurones in the mucosa (Baraniuk JN, 1998), a great proportion of the released NA was taken up into the neurones by Uptake<sub>1</sub> and metabolized into DHPG by MAO (Eisenhofer G *et al.*, 1992). In addition, it has been reported that NA is unable to cross membranes to any great degree without the aid of specific transporters (Horvath G *et al.*, 2003). In an *in vitro* study investigating the contribution of neuronal and extraneuronal catecholamine uptake and metabolism in rabbit nasal mucosa, the majority of <sup>3</sup>H-NA was converted into <sup>3</sup>H-DHPG (de la Lande IS *et al.*, 1987). If neuronal mechanisms of adrenergic uptake and metabolism contribute more to the control of sympathetic activity in the nasal mucosa than extraneuronal mechanisms, it could explain the lack of MHPG peaks in the lavages: DHPG is converted by the extraneuronal COMT into MHPG.

Finally, it is interesting that the levels of DHPG (and adrenaline) were not significantly different between the initial and final nasal lavages. Higher levels of mediators tend to be recorded in initial lavages, and this is thought to be due to a gradual build up prior to the lavage (Naclerio RM *et al.*, 1983). This is usually overcome by performing 3-5 lavages and using the final lavage as a measure of the basal level of mediator release. However, this appears not to be necessary for DHPG levels in nasal lavage, suggesting that DHPG does not accumulate in the nasal mucosa and so DHPG may prove to be a sensitive tool for measuring changes in sympathetic activity. DHPG has been previously used as a measure of regional sympathetic activity (Eisenhofer G *et al.*, 1992; Esler M, 1993): DHPG outflow increases with increased sympathetic nerve firing rates.

In conclusion, although NA levels may be low, high levels of the NA-metabolite DHPG have been demonstrated in human nasal lavages. This observation is in agreement with circumstantial evidence in the literature that suggests a role of the sympathetic nervous system in the control of nasal patency. If nasal patency is maintained to some degree by sympathetically-released NA, interference of adrenergic pathways should cause nasal blockage.

Lidocaine, following bilateral nasal administration as an aerosol, 4mg, had no effect on the area under the normalised Amin. versus time curve measured over a period of 15 minutes (AUC), although, as shown in Figure 6.19, lidocaine did cause significant anaesthesia of the nasal mucosa. This observation is in agreement with Dear (1996) and suggests that inhibition of neuronal activity does not affect nasal patency, although another study demonstrated ipsilateral nasal blockage following unilateral challenge with a local anaesthetic (Eccles R, 2000). There is an abundance of evidence that shows sympathetic neurones are sensitive to inhibition by local anaesthetics (Polwin W *et al.*, 1987; Ellenbogen KA *et al.*, 1992; Pietruck C *et al.*, 2003), but local anaesthetics also affect other excitable tissues, such as vascular smooth muscle (Covino BG, 1987). Indeed, the effect of local anaesthetics on vascular smooth muscle is dose-dependent, with low doses producing vasoconstriction and higher doses, vasodilation. This biphasic action on vascular smooth muscle may explain the lack of nasal blockage following lidocaine challenge: the effect on nasal patency of a decrease in sympathetic activity (and NA release) could be functionally antagonized by direct vasoconstriction caused by lidocaine. In addition, local anaesthetics may inhibit the activity of parasympathetic neurones. As discussed in Chapter 1.7.2, the role of ACh and VIP in the control of nasal patency is not clear. However, a reduction of NA release by lidocaine may be counterbalanced by a decrease in the release of vasodilatory parasympathetic substances, thus no change in patency would arise. Finally, it is possible that, following nasal challenge with lidocaine, the exposure of the local anaesthetic to the nasal sensory neurones is different to its exposure to sympathetic neurones. Thus the sensation of anaesthesia may not adequately demonstrate an effective lidocaine concentration at sympathetic neurones.

Clonidine, following bilateral nasal administration as an aerosol, 100µg, caused a significant increase in the area under the normalised Amin. versus time curve

measured over a period of 15 minutes (AUC). This increase in AUC represents an increase in nasal patency, as a result of increased vasoconstriction of nasal blood vessels. This vasoconstriction may be due to activation of postsynaptic  $\alpha_2$ -adrenoceptors on nasal blood vessels. However, as clonidine is a partial agonist at  $\alpha_2$ -adrenoceptors (Stump DC *et al.*, 1983; Chapleo CB *et al.*, 1989), it is possible for clonidine to act as an antagonist in the presence of a high concentration of a full agonist. As already demonstrated in this chapter, NA is probably released from nasal sympathetic neurones in a continuous manner. Therefore, clonidine may antagonize NA at presynaptic  $\alpha_2$ -adrenoceptors. Activation of these autoreceptors by NA inhibits further NA release, as shown in Figure 6.1. Thus clonidine, by antagonizing this pathway, may increase the release of NA, thereby producing more vasoconstriction and, hence, increasing nasal patency. Without selective agonists for pre- and postsynaptic  $\alpha_2$ -adrenoceptors, it is impossible, using functional studies, to elucidate the mechanism of clonidine-induced nasal decongestion. However, it may be possible to analyze the effect of clonidine on NA or DHPG levels in nasal lavages.

Corynanthine, following bilateral nasal administration as an aerosol, 2mg, caused a significant decrease in the area under the normalised Amin. versus time curve measured over a period of 15 minutes (AUC). This decrease in AUC represents a decrease in nasal patency or, alternatively, increased nasal blockage. Corynanthine is a potent antagonist with high selectivity for  $\alpha_1$ -adrenoceptors (Doxey JC *et al.*, 1984), thus it seems likely that the corynanthine-induced nasal blockage is due to the removal of vasoconstriction caused by endogenous NA. This observation suggests that NA, under resting conditions, actively maintains the patency of the nasal airways in normal, non-atopic humans, via activation of postsynaptic  $\alpha_1$ -adrenoceptors. Clearly vasoconstriction due to basally-released NA counterbalances a tendency for the nasal blood vessels to dilate. The source of this vasodilation is not known: inhibitors of NO and PGI<sub>2</sub> synthesis have no effect on resting nasal patency (McLean JA *et al.*, 1983; Dear JW *et al.*, 1996a); and, in addition, vidian neurectomy and muscarinic antagonists fail to increase nasal patency (Konno A *et al.*, 1979; Barody FM *et al.*, 1994). It is possible that the vasodilation may be due to the hydrostatic pressure of the blood on the vessel walls.

Corynanthine causes nasal blockage by inhibiting endogenous NA at  $\alpha_1$ -adrenoceptors, which is in agreement with the reported side-effect of nasal congestion following treatment with  $\alpha_1$ -adrenoceptor antagonists (Moyer JH *et al.*, 1953; Moser M, 1958; Caine M *et al.*, 1981; Kirby RS, 1999). However, the increase in nasal patency following clonidine challenge (Figure 6.20) suggests that activation postsynaptic  $\alpha_2$ -adrenoceptors may also produce vasoconstriction. The contribution of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors to nasal blood vessel vasoconstriction varies between species: the  $\alpha_2$ -adrenoceptor appears to play a more prominent role in mediating vasoconstriction in the cat than in the rat (Malm L, 1977; Kristiansen AB *et al.*, 1993; Kawarai M *et al.*, 2001; Mcleod RL *et al.*, 2001). In man, drugs such as oxymetazoline, xylometazoline and phenylephrine are prescribed as decongestants and have been shown to cause vasoconstriction in human nasal blood vessels (Cole P *et al.*, 1983; Ichimura K *et al.*, 1988; Johannssen V *et al.*, 1997; Bickford L *et al.*, 1999). However, these drugs are activators of both  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors (with phenylephrine partially selective for  $\alpha_1$ -adrenoceptors and oxymetazoline and xylometazoline partially selective for  $\alpha_2$ -adrenoceptors) (U'Prichard DC *et al.*, 1977; Hirasawa A *et al.*, 1993); in fact, in one study, Ichimura & Chow (1988) showed that the vasoconstriction caused by oxymetazoline (considered by the authors to be a selective  $\alpha_2$ -adrenoceptor agonist) was inhibited by prazosin, the selective  $\alpha_1$ -adrenoceptor antagonist. Thus it is not clear from these  $\alpha$ -adrenoceptor agonist studies whether  $\alpha_2$ -adrenoceptors play a role in the control of nasal patency. Both Ichimura & Chow (1988) and Johannssen *et al.* (1997) suggested that  $\alpha_1$ -adrenoceptor antagonists could abolish the majority of NA-induced contractions, but that  $\alpha_2$ -adrenoceptors probably mediated some sympathetic actions. This hypothesis has recently been bolstered by a report that demonstrated *in vitro* electric field stimulation-induced vasoconstriction of human nasal mucosa was only abolished by a combination of prazosin and yohimbine, the  $\alpha_2$ -adrenoceptor antagonist (prazosin alone inhibited the majority of the vasoconstriction) (Varty LM *et al.*, 2004). Therefore, although the  $\alpha_1$ -adrenoceptor appears to mediate the lion's share of the vasoconstriction caused by basally-released NA, postsynaptic  $\alpha_2$ -adrenoceptors may also contribute.

There is little evidence in the literature to suggest a role of  $\beta$ -adrenoceptors in the control of nasal patency (Svensson G *et al.*, 1980; Nagai M *et al.*, 1984), although  $\beta$ -adrenoceptor levels appear to be lower in atopic individuals (van Megen YJ *et al.*,



1991) and stimulation of  $\beta$ -adrenoceptors in the sphenopalatine veins of dogs is thought to promote decongestion by facilitating the draining of engorged sinusoids (Wang M *et al.*, 2003). Although NPY causes nasal decongestion when applied to human nasal mucosa *in vivo* (Baraniuk JN *et al.*, 1992), NPY antagonists have yet to be used *in vivo* in humans to assess the contribution of basally-released NPY in the control of nasal patency. The nasal vascular responses to ATP, another sympathetic-derived co-transmitter, have also been studied (Lacroix JS *et al.*, 1988a). ATP is thought to contribute to the sympathetic control of nasal patency in cats and dogs (Lacroix JS *et al.*, 1994b), but, again, there has not been much investigation of this substance in human nasal mucosa. Given the lack of evidence of the actions of endogenous sympathetic-derived co-transmitters, it is difficult to describe accurately the complete role of the sympathetic nervous system in the control of nasal patency.

## 6.7 Summary

- 1) Nanomolar levels of NA were measured in nasal lavages using HPLC-ECD. NA levels were stabilized by cold, dark and acidic conditions.
- 2) High levels of DHPG, the NA-metabolite, were also measured in nasal lavages using HPLC-ECD.
- 3) Clonidine, the partial  $\alpha_2$ -adrenoceptor agonist, 100 $\mu$ g, induced a significant increase in the patency of the nasal airways of normal individuals. This result is thought to be due to the activation of postsynaptic vascular  $\alpha_2$ -adrenoceptors; or due to the inhibition of presynaptic sympathetic  $\alpha_2$ -adrenoceptors, thus promoting increased release of endogenous NA.
- 4) Corynanthine, the  $\alpha_1$ -adrenoceptor antagonist, 2mg, induced a significant decrease in the patency of the nasal airways of normal individuals, observed as an increase in nasal blockage.
- 5) The data indicate that there is basal release of NA from nasal sympathetic neurones in normal individuals, and that inhibition of endogenous NA causes nasal blockage. Therefore, in the nasal mucosa of normal, non-atopic, individuals, the sympathetic nervous system actively maintains nasal patency via the activation of  $\alpha_1$ -adrenoceptors (and possibly  $\alpha_2$ -adrenoceptors) by basally-released NA.

# CHAPTER 7

## THE ROLE OF THE HISTAMINE H<sub>3</sub> RECEPTOR IN HISTAMINE-INDUCED NASAL BLOCKAGE

### 7.1 Introduction

As demonstrated in Chapters 3 and 4, histamine causes nasal blockage in humans via H<sub>1</sub> and H<sub>2</sub> receptors. However, pretreatment with a high dose combination of H<sub>1</sub> and H<sub>2</sub> antagonists was unable to abolish the histamine-induced nasal blockage completely, thus indicating that histamine was causing nasal blockage by, at least, one other mechanism. This unknown mechanism is of particular interest as it may explain, at least in part, the apparent ineffectiveness of H<sub>1</sub> and H<sub>2</sub> antagonists in reducing pollen-induced nasal blockage, as shown in Chapter 5. If histamine, released after mast cell degranulation, is responsible for mediating pollen-induced nasal blockage, then perhaps this unknown histaminergic pathway is the mechanism by which pollen-induced nasal blockage is mediated.

Recent research has identified a role of H<sub>3</sub> receptors in the control of nasal blockage (McLeod RL *et al.*, 1999b; Varty L *et al.*, 2002; Varty LM *et al.*, 2004). H<sub>3</sub> antagonists, in combination with H<sub>1</sub> antagonists, have been shown to reduce the nasal blockage caused by compound 48/80, the mast cell degranulator, in an *in vivo* feline model of allergic rhinitis (McLeod RL *et al.*, 1999b; McLeod RL *et al.*, 2003). In addition, H<sub>3</sub> receptor mRNA has been demonstrated in human nasal mucosa and R- $\alpha$ -methylhistamine, the H<sub>3</sub> agonist, inhibited the *in vitro* electric field stimulation-induced vasoconstriction of human nasal mucosa (Varty LM *et al.*, 2004). In this chapter, the contribution of H<sub>3</sub> receptors to the nasal blockage caused by histamine, after it has been sprayed into the nasal cavities of normal, non-atopic, human subjects, has been investigated.

The H<sub>3</sub> ligands used in this study were first described by Arrang *et al.* (1987). R- $\alpha$ -methylhistamine is a potent H<sub>3</sub> agonist, with some potency at H<sub>4</sub> receptors but with virtually no activity at H<sub>1</sub> or H<sub>2</sub> receptors (van der Goot H *et al.*, 2000). S- $\alpha$ -

methylhistamine has an affinity for the H<sub>3</sub> receptor a hundred times less than its stereoisomer, R- $\alpha$ -methylhistamine (Arrang JM *et al.*, 1987). Both are, however, full agonists. Thioperamide is a potent and selective H<sub>3</sub>/H<sub>4</sub> antagonist (K<sub>i</sub> of 1-10nM), with negligible affinity for H<sub>1</sub> receptors, H<sub>2</sub> receptors, adrenoceptors and muscarinic receptors (Arrang JM *et al.*, 1987; van der Goot H *et al.*, 2000).

## 7.2 Experimental protocol

In the following double-blind experiments, healthy non-atopic human volunteers were used as subjects.

### 7.2.1 The effect of topical histamine H<sub>3</sub> agonist, R- $\alpha$ -methylhistamine, on nasal patency

The aim of this experiment was to investigate the effect of an H<sub>3</sub> agonist on nasal patency, as measured by acoustic rhinometry. Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline or R- $\alpha$ -methylhistamine, 100, 300 or 600 $\mu$ g. Each subject received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The doses and time-course used were based upon comparisons with histamine and the published potency of the H<sub>3</sub> agonist, R- $\alpha$ -methylhistamine (Arrang JM *et al.*, 1987). The protocol is summarized in Figure 7.1.

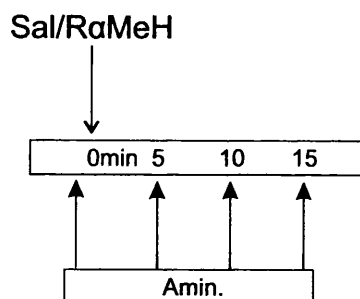


Figure 7.1: Protocol for investigating the effect of R- $\alpha$ -methylhistamine on nasal patency. Sal = saline, RαMeH = R- $\alpha$ -methylhistamine.

### 7.2.2 The effect of topical histamine H<sub>3</sub> agonist, S- $\alpha$ -methylhistamine, on nasal patency

The aim of this experiment was to assess whether S- $\alpha$ -methylhistamine would cause less nasal blockage than the same dose of the more potent stereoisomer R- $\alpha$ -methylhistamine. Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline, R- $\alpha$ -methylhistamine, 600 $\mu$ g, or S- $\alpha$ -methylhistamine, 600 $\mu$ g. Each subject

received each treatment in a random order, with at least 24 hours between treatments. Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 7.2.

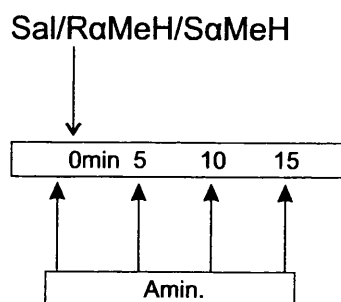


Figure 7.2: Protocol for investigating the effect of S- $\alpha$ -methylhistamine on nasal patency. Sal = saline, R $\alpha$ MeH = R- $\alpha$ -methylhistamine, S $\alpha$ MeH = S- $\alpha$ -methylhistamine.

### 7.2.3 The effect of topical $\alpha_1$ -adrenoceptor antagonist, corynanthine, on R- $\alpha$ -methylhistamine- and bradykinin-induced nasal blockage

The aim of this experiment was to investigate the degree to which corynanthine, a selective  $\alpha_1$ -adrenoceptor antagonist, would augment the nasal blockage caused by R- $\alpha$ -methylhistamine and bradykinin. Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline, R- $\alpha$ -methylhistamine, 300 or 600 $\mu$ g, or bradykinin, 100 or 200 $\mu$ g, with or without corynanthine, 2mg. Subjects received the following 10 single aerosol spray treatments in a random order, with at least 24 hours between each treatment:

- i) Saline
- ii) R- $\alpha$ -methylhistamine, 300 $\mu$ g
- iii) R- $\alpha$ -methylhistamine, 600 $\mu$ g
- iv) Bradykinin, 100 $\mu$ g
- v) Bradykinin, 200 $\mu$ g
- vi) Corynanthine, 2mg
- vii) R- $\alpha$ -methylhistamine, 300 $\mu$ g and corynanthine, 2mg
- viii) R- $\alpha$ -methylhistamine, 600 $\mu$ g and corynanthine, 2mg
- ix) Bradykinin, 100 $\mu$ g and corynanthine, 2mg

x) Bradykinin, 200µg and corynanthine, 2mg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 13 subjects were included in this study. The dose of corynanthine used was based upon the published topical dose used in a study investigating the effect of corynanthine on intraocular pressure in man (Serle JB *et al.*, 1985) and previous studies in Chapter 6. The dose of bradykinin was based on pilot studies and previously published studies investigating the effect of topical bradykinin on nasal patency (Austin CE *et al.*, 1994a). The protocol is summarized in Figure 7.3.

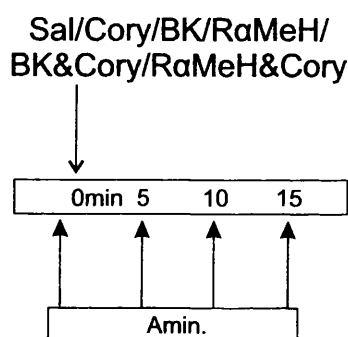


Figure 7.3: Protocol for investigating the effects of corynanthine on R-α-methylhistamine- and bradykinin-induced nasal blockage. Sal = saline, Cory = corynanthine, BK = bradykinin, RαMeH = R-α-methylhistamine.

#### 7.2.4 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, and oral histamine H<sub>2</sub> antagonist, ranitidine, on R-α-methylhistamine-induced nasal blockage

The aim of this experiment was to investigate the role of H<sub>1</sub> and H<sub>2</sub> receptors in R-α-methylhistamine-induced nasal blockage. Subjects received oral cetirizine, 20mg, or oral ranitidine, 75mg, or oral placebo. 2 hours later, subjects received, by 100µl aerosol into each nostril, saline or R-α-methylhistamine, 600µg. Each subject received the following 4 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline
- ii) Placebo; R-α-methylhistamine, 600µg
- iii) Cetirizine, 20mg; R-α-methylhistamine, 600µg
- iv) Ranitidine, 75mg; R-α-methylhistamine, 600µg

Immediately prior to, and 5, 10 and 15 minutes after nasal challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The doses of cetirizine and ranitidine were based on the effective antagonist doses demonstrated in previous studies (described in Chapters 3 & 4). The protocol is summarized in Figure 7.4.

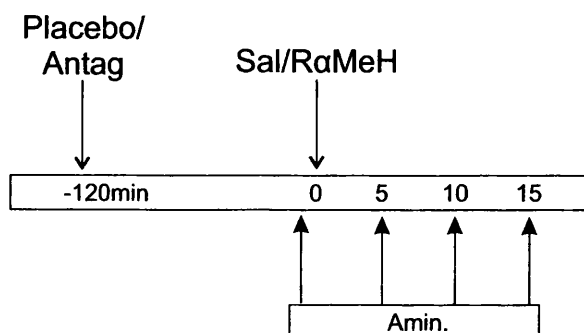


Figure 7.4: Protocol for investigating the effect of oral H<sub>1</sub> and H<sub>2</sub> antagonists on R-α-methylhistamine-induced nasal blockage. Antag = antagonist, Sal = saline, RαMeH = R-α-methylhistamine.

### 7.2.5 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on nasal patency

The aim of this experiment was investigate the effect of an H<sub>3</sub> antagonist on nasal patency, as measured by acoustic rhinometry. The minimum cross-sectional area (Amin.) of the nasal airways was measured, for each subject, using acoustic rhinometry. Subjects then received, by 100μl aerosol into each nostril, either saline or thioperamide, 700μg. Either 15 or 45 minutes later, Amin. was then re-measured using acoustic rhinometry. Each subject received each treatment in a random order, with at least 24 hours between treatments. 8 subjects were included in this study. The dose used was based upon comparisons with topical H<sub>1</sub> antagonist, chlorpheniramine, and the published potency of the H<sub>3</sub> antagonist, thioperamide (Arrang JM *et al.*, 1987). The time-course used was based upon *in vitro* and *in vivo* studies using thioperamide (McLeod RL *et al.*, 1999b; Varty L *et al.*, 2002). The protocol is summarized in Figure 7.5.



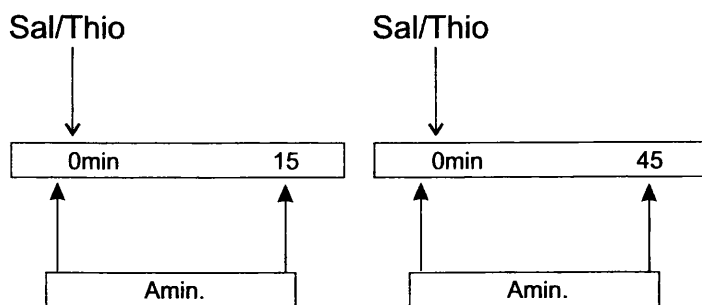


Figure 7.5: Protocol for investigating the effect of thioperamide on nasal patency. Sal = saline, Thio = thioperamide.

### 7.2.6 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on R- $\alpha$ -methylhistamine-induced nasal blockage

The aim of this experiment was to investigate whether the nasal blockage caused by the H<sub>3</sub> agonist, R- $\alpha$ -methylhistamine, is mediated via H<sub>3</sub> receptors. Subjects received, by 100 $\mu$ l aerosol into each nostril, either saline or thioperamide, 700 $\mu$ g. 45 minutes later, subjects received, by 100 $\mu$ l aerosol into each nostril, saline or R- $\alpha$ -methylhistamine, 600 $\mu$ g. Each subject received the following 3 treatments in a random order, with at least 24 hours between each treatment:

- i) Saline; saline
- ii) Saline; R- $\alpha$ -methylhistamine, 600 $\mu$ g
- iii) Thioperamide, 700 $\mu$ g; R- $\alpha$ -methylhistamine, 600 $\mu$ g

Immediately prior to, and 5, 10 and 15 minutes after saline/R- $\alpha$ -methylhistamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 10 subjects were included in this study. The protocol is summarized in Figure 7.6.

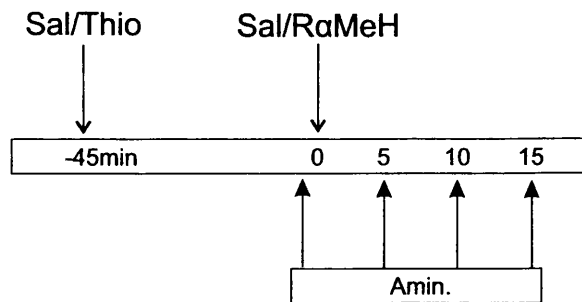


Figure 7.6: Protocol for investigating the effect of thioperamide on R-α-methylhistamine-induced nasal blockage. Sal = saline, Thio = thioperamide, RαMeH = R-α-methylhistamine.

### 7.2.7 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on histamine-induced nasal blockage

The aim of this experiment is to investigate whether the nasal blockage caused by histamine is mediated via H<sub>3</sub> receptors. Subjects received, by 100μl aerosol into each nostril, either saline or thioperamide, 700μg. 45 minutes later, subjects received, by 100μl aerosol into each nostril, saline or histamine, 400μg. Each subject received the following 3 treatments in a random order, with at least 24 hours between each treatment:

- i) Saline; saline
- ii) Saline; histamine, 400μg
- iii) Thioperamide, 700μg; histamine, 400μg

Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 8 subjects were included in this study. The protocol is summarized in Figure 7.7.

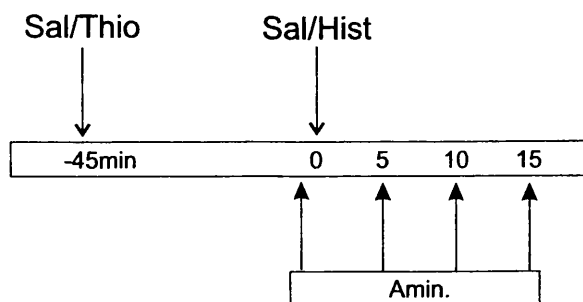


Figure 7.7: Protocol for investigating the effect of thioperamide (45 minute incubation) on histamine-induced nasal blockage. Sal = saline, Thio = thioperamide, Hist = histamine.

This study was repeated, using the same 8 subjects, with the saline/thioperamide aerosol administered 15 minutes prior to saline/histamine challenge. The protocol is summarized in Figure 7.8.

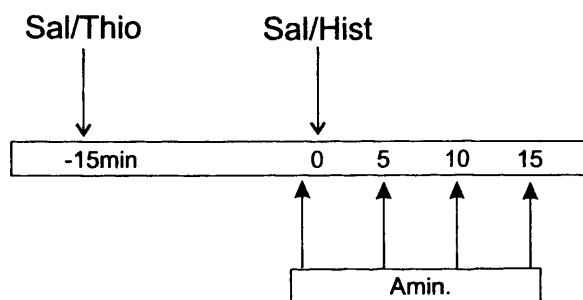


Figure 7.8: Protocol for investigating the effect of thioperamide (15 minute incubation) on histamine-induced nasal blockage. Sal = saline, Thio = thioperamide, Hist = histamine.

### 7.2.8 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>1</sub> antagonist, cetirizine

The aim of this experiment is to investigate whether the residual nasal blockage caused by histamine in the presence of an H<sub>1</sub> antagonist is sensitive to an H<sub>3</sub> antagonist. Subjects received oral cetirizine, 20mg, or oral placebo. 75 minutes later, subjects received, by 100µl aerosol into each nostril, saline or thioperamide, 700µg. 45 minutes later, subjects then received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 4 treatments in a random order, with at least 48 hours between each treatment:

i) Placebo; saline; saline

- ii) Placebo; saline; histamine, 400µg
- iii) Cetirizine, 20mg; saline; histamine, 400µg
- iv) Cetirizine, 20mg; thioperamide, 700µg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 15 subjects were included in this study. The protocol is summarized in Figure 7.9.

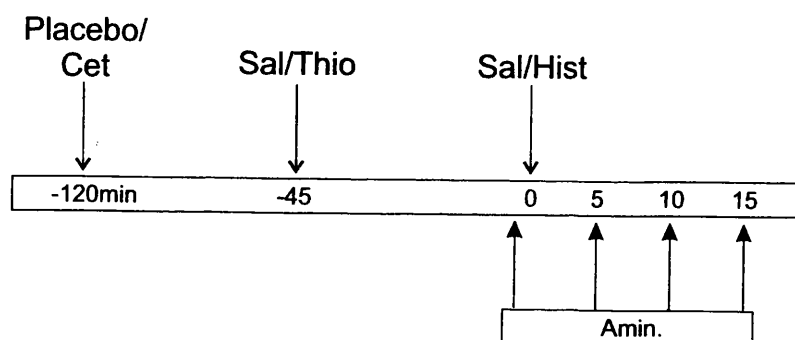


Figure 7.9: Protocol for investigating the effect of thioperamide on the reduction of histamine-induced nasal blockage by oral H<sub>1</sub> antagonist, cetirizine. Cet = cetirizine, Sal = saline, Thio = thioperamide, Hist = histamine.

### 7.2.9 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination with (i) topical histamine H<sub>3</sub> antagonist, thioperamide, (ii) oral histamine H<sub>2</sub> antagonist, ranitidine, or (iii) both thioperamide and ranitidine, on histamine-induced nasal blockage

The aim of this experiment was to investigate whether an H<sub>3</sub> antagonist reduces histamine-induced nasal blockage when in combination with an H<sub>1</sub> antagonist, or with both an H<sub>1</sub> antagonist and an H<sub>2</sub> antagonist. Subjects received, alone or in combination, oral cetirizine, 20mg, oral ranitidine, 150mg, or oral placebo. 75 minutes later, subjects received, by 100µl aerosol into each nostril, saline or thioperamide, 700µg. 45 minutes later, subjects then received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 6 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline; saline
- ii) Placebo; saline; histamine, 400µg

- iii) Cetirizine, 20mg; saline; histamine, 400µg
- iv) Cetirizine, 20mg; thioperamide, 700µg; histamine, 400µg
- v) Cetirizine, 20mg and ranitidine 150mg; saline; histamine, 400µg
- vi) Cetirizine, 20mg and ranitidine 150mg; thioperamide, 700µg; histamine, 400µg

Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 15 subjects were included in this study. The protocol is summarized in Figure 7.10.

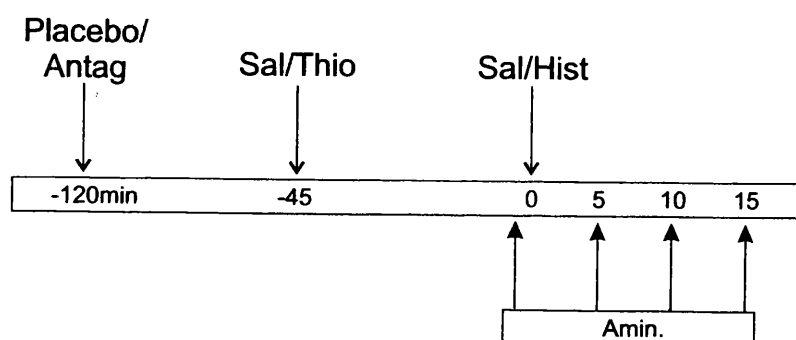


Figure 7.10: Protocol for investigating the effect of oral cetirizine, alone or in combination with (i) topical thioperamide, (ii) oral ranitidine, or (iii) both thioperamide and ranitidine, on histamine-induced nasal blockage. Antag = oral antagonist, Sal = saline, Thio = thioperamide, Hist = histamine.

#### 7.2.10 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, and oral histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination, on histamine-induced nasal blockage

The aim of this experiment is to investigate whether repeated topical administration of an H<sub>3</sub> antagonist reduces the nasal blockage caused by histamine, with or without pretreatment with an H<sub>1</sub> antagonist. Subjects received oral cetirizine, 20mg, or oral placebo. Subjects then received three administrations, by 100µl aerosol into each nostril, of saline or thioperamide, 700µg, twenty minutes apart, one hour after the oral placebo/antagonist. 20 minutes after the final saline/thioperamide aerosol spray, the subjects finally received, by 100µl aerosol into each nostril, saline or histamine, 400µg. Each subject received the following 5 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline (3 times); saline
- ii) Placebo; saline (3 times); histamine, 400 $\mu$ g
- iii) Placebo; thioperamide, 700 $\mu$ g (3 times); histamine, 400 $\mu$ g
- iv) Cetirizine, 20mg; saline (3 times); histamine, 400 $\mu$ g
- v) Cetirizine, 20mg; thioperamide, 700 $\mu$ g (3 times); histamine, 400 $\mu$ g

Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area ( $A_{min}$ ) of the nasal airways was measured using acoustic rhinometry. 15 subjects were included in this study. The protocol is summarized in Figure 7.11.

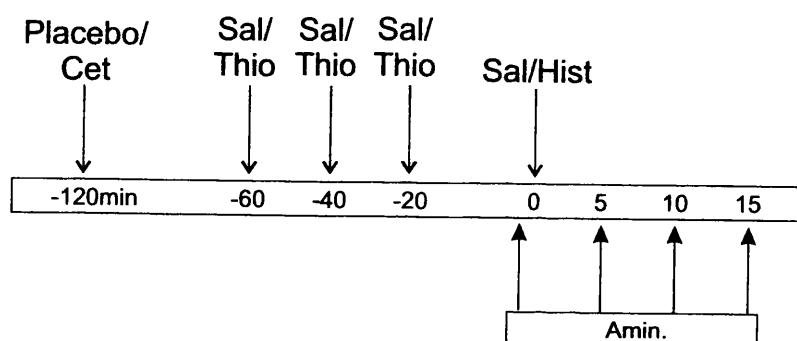


Figure 7.11: Protocol for investigating the effect of topical thioperamide and oral cetirizine, alone and in combination, on histamine-induced nasal blockage. Cet = cetirizine, Sal = saline, Thio = thioperamide, Hist = histamine.

### 7.2.11 The effect of topical histamine $H_3$ antagonist, thioperamide, and oral histamine $H_1$ antagonist, cetirizine, alone or in combination, on the nasal blockage caused by a high dose of histamine.

The aim of this experiment is to assess whether a higher dose of histamine causes nasal blockage that is sensitive to repeated topical administration of an  $H_3$  antagonist, with or without pretreatment with an  $H_1$  antagonist. Subjects received oral cetirizine, 20mg, or oral placebo. Subjects then received 3 administrations, by 100 $\mu$ l aerosol into each nostril, of saline or thioperamide, 700 $\mu$ g, 20 minutes apart, one hour after the oral placebo/antagonist. 20 minutes after the final saline/thioperamide aerosol spray, the subjects finally received, by 100 $\mu$ l aerosol into each nostril, saline or histamine, 1000 $\mu$ g. Each subject received the following 5 treatments in a random order, with at least 48 hours between each treatment:

- i) Placebo; saline (3 times); saline
- ii) Placebo; saline (3 times); histamine, 1000 $\mu$ g
- iii) Placebo; thioperamide, 700 $\mu$ g (3 times); histamine, 1000 $\mu$ g
- iv) Cetirizine, 20mg; saline (3 times); histamine, 1000 $\mu$ g
- v) Cetirizine, 20mg; thioperamide, 700 $\mu$ g (3 times); histamine, 1000 $\mu$ g

Immediately prior to, and 5, 10 and 15 minutes after saline/histamine challenge, the minimum cross-sectional area (Amin.) of the nasal airways was measured using acoustic rhinometry. 15 subjects were included in this study. The protocol is summarized in Figure 7.12.

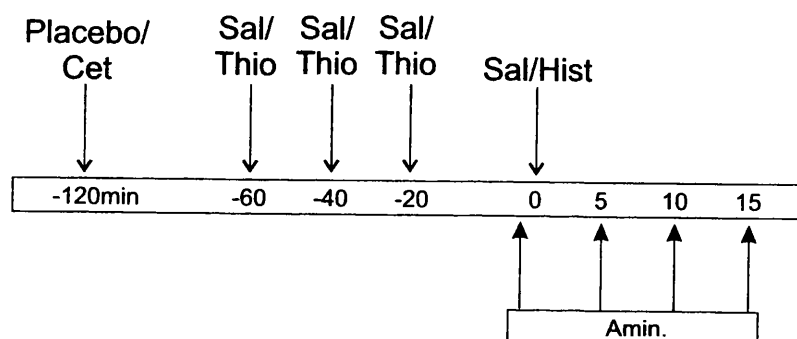


Figure 7.12: Protocol for investigating the effect of topical thioperamide and oral cetirizine, alone and in combination, on the nasal blockage caused by a high dose of histamine. Cet = cetirizine, Sal = saline, Thio = thioperamide, Hist = histamine.

### 7.2.12 Data Analysis

Nasal patency was measured using acoustic rhinometry, as described in Chapter 2. Amin. was recorded immediately prior to and 5, 10 and 15 minutes after nasal challenge. Amin. values were normalised to the Amin. value recorded prior to nasal challenge. For each challenge (except in Chapter 7.2.5), a normalised Amin. versus time curve was plotted, and the area under the curve (AUC) calculated. In Chapter 7.2.5, as different time-points were recorded, the AUC was not calculated. The rhinometry data are presented either as mean values, together with s.e.mean; or as individual data. The appropriate non-parametric statistical test is given with each data set.  $p < 0.05$  is taken as significant.

## 7.3 Results

### 7.3.1 The effect of topical histamine H<sub>3</sub> agonist, R- $\alpha$ -methylhistamine, on nasal patency

Application, by aerosol into each nostril, of R- $\alpha$ -methylhistamine, 300 and 600 $\mu$ g, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge, as shown in Figure 7.13.

### 7.3.2 The effect of topical histamine H<sub>3</sub> agonist, S- $\alpha$ -methylhistamine, on nasal patency

Application, by aerosol into each nostril, of R- $\alpha$ -methylhistamine, 600 $\mu$ g, caused a significant reduction in AUC as compared to saline and S- $\alpha$ -methylhistamine ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Application, by aerosol into each nostril, of S- $\alpha$ -methylhistamine, 600 $\mu$ g, had no effect on AUC as compared to saline ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 7.14).

### 7.3.3 The effect of topical $\alpha_1$ -adrenoceptor antagonist, corynanthine, on R- $\alpha$ -methylhistamine- and bradykinin-induced nasal blockage

From *in vitro* studies, it was hypothesized that R- $\alpha$ -methylhistamine mediated its nasal response via inhibition of the basal release of noradrenaline (Varty L *et al.*, 2002; Varty LM *et al.*, 2004). If R- $\alpha$ -methylhistamine causes nasal blockage via this mechanism, then the reduction in AUC caused by R- $\alpha$ -methylhistamine and corynanthine, the selective  $\alpha_1$ -adrenoceptor antagonist, should not be additive.

Application, by aerosol into each nostril, of either bradykinin, 100 $\mu$ g or 200 $\mu$ g, or R- $\alpha$ -methylhistamine, 600 $\mu$ g, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. However, application, by aerosol into each nostril, of R- $\alpha$ -methylhistamine, 300 $\mu$ g, had no significant effect on AUC as compared to saline ( $p > 0.05$ , Wilcoxon sign-rank test). Application, by



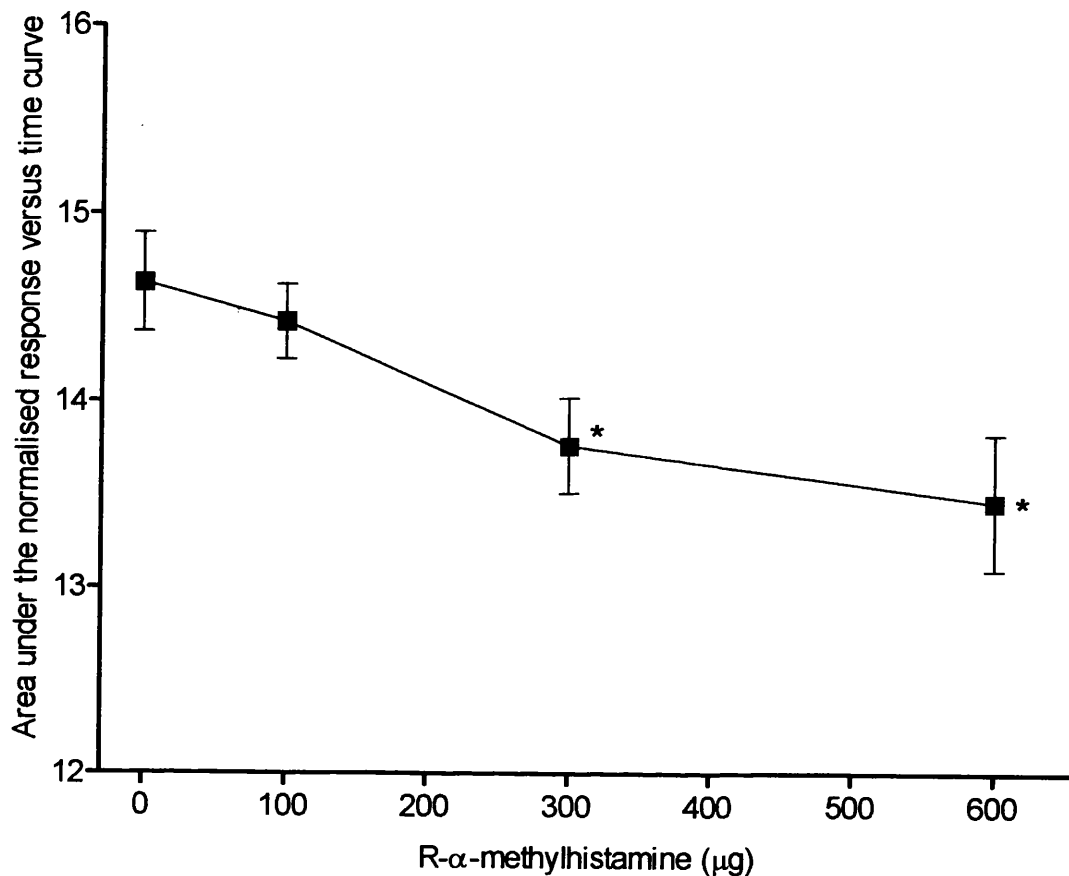


Figure 7.13: Dose-response curve for the action of R-α-methylhistamine on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of R-α-methylhistamine as an aerosol, at the dose shown, into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.693cm<sup>2</sup>, s.e.mean of 0.039cm<sup>2</sup>). Data are means from 8 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

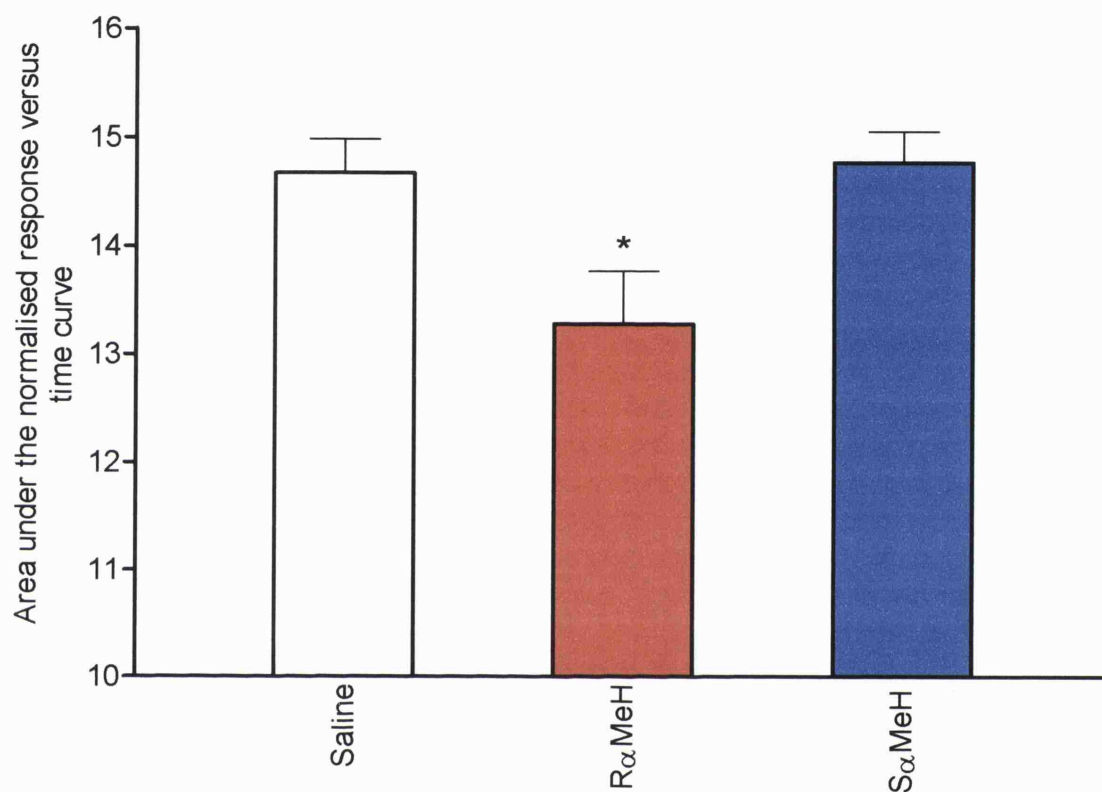


Figure 7.14: The effect of R- $\alpha$ -methylhistamine [R $\alpha$ MeH] and S- $\alpha$ -methylhistamine [S $\alpha$ MeH] on the area under the normalized Amin. versus time curve measured over a period of 15 minutes following the administration of R- $\alpha$ -methylhistamine, 600 $\mu$ g, or S- $\alpha$ -methylhistamine, 600 $\mu$ g, as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.580cm<sup>2</sup>, s.e.mean of 0.041cm<sup>2</sup>). Data are means from 8 people. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

aerosol into each nostril, of corynanthine, 2mg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 7.15).

A combination of corynanthine, 2mg, with either bradykinin, 100µg or 200µg, or R-α-methylhistamine, 300µg, caused a significantly greater reduction in AUC as compared to without corynanthine ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to greater nasal blockage measured over the 15 minutes after nasal challenge. A combination of corynanthine, 2mg, with R-α-methylhistamine, 600µg, caused a reduction in AUC that was not significantly greater than the reduction in AUC caused by R-α-methylhistamine, 600µg ( $p > 0.05$ , Wilcoxon sign-rank test) (Figure 7.15).

The difference in AUC between corynanthine, 2mg, alone and saline challenge was calculated for each subject ( $\Delta$  area under the normalised Amin. versus time curve ( $\Delta$ AUC)). The  $\Delta$ AUC (difference between with and without corynanthine, 2mg) was also calculated for bradykinin, 100 and 200µg, and R-α-methylhistamine, 300 and 600µg. The calculated  $\Delta$ AUC for each subject are plotted in Figure 7.16.

Bradykinin, 100 and 200µg, and R-α-methylhistamine, 300µg, had no effect on the  $\Delta$  area under the normalised Amin. versus time curve ( $\Delta$ AUC) caused by corynanthine, 2mg, as compared to saline ( $p > 0.05$ , Wilcoxon sign-rank test). However, R-α-methylhistamine, 600µg, significantly reduced the  $\Delta$ AUC caused by corynanthine, 2mg, as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test). This shows that the difference in AUC between nasal challenges with corynanthine and without corynanthine is significantly less when the corynanthine is in combination with the high dose of R-α-methylhistamine (600µg).

#### **7.3.4 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, and oral histamine H<sub>2</sub> antagonist, ranitidine, on R-α-methylhistamine-induced nasal blockage**

Application, by aerosol into each nostril, of R-α-methylhistamine, 600µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral cetirizine, 20mg, or ranitidine, 75mg, both given 2 hours prior to nasal challenge, had no effect on the reduction in AUC

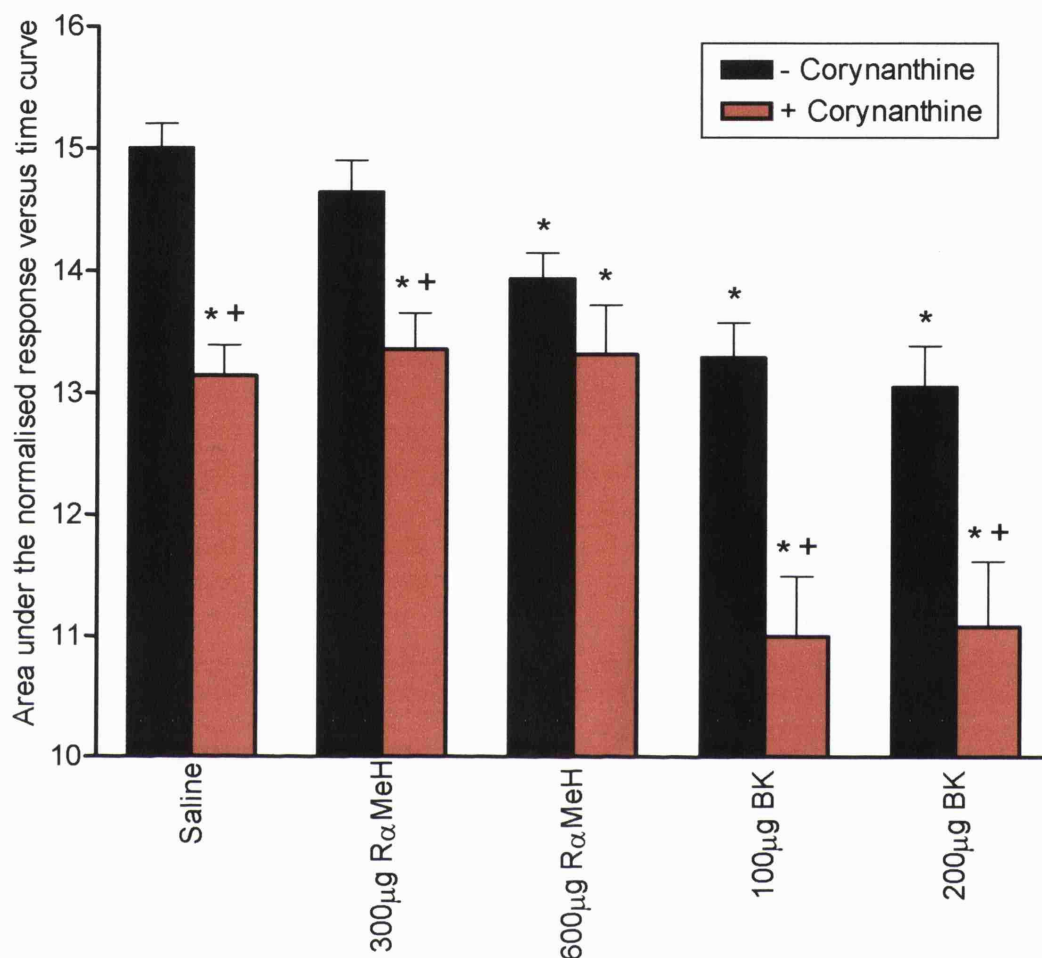


Figure 7.15: The effect of corynanthine, 2mg, given as an aerosol into each nostril, on the nasal blockage caused by either R- $\alpha$ -methylhistamine [R $\alpha$ MeH] or bradykinin [BK]. Both R- $\alpha$ -methylhistamine, 300µg and 600µg, and bradykinin, 100µg and 200µg, were administered as aerosols into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.797cm<sup>2</sup>, s.e.mean of 0.024cm<sup>2</sup>). The data are the means from 13 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the nasal challenge. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control (p<0.05, Wilcoxon sign-rank test). + Significant increase in nasal blockage with corynanthine as compared to without corynanthine p<0.05, Wilcoxon sign-rank test).

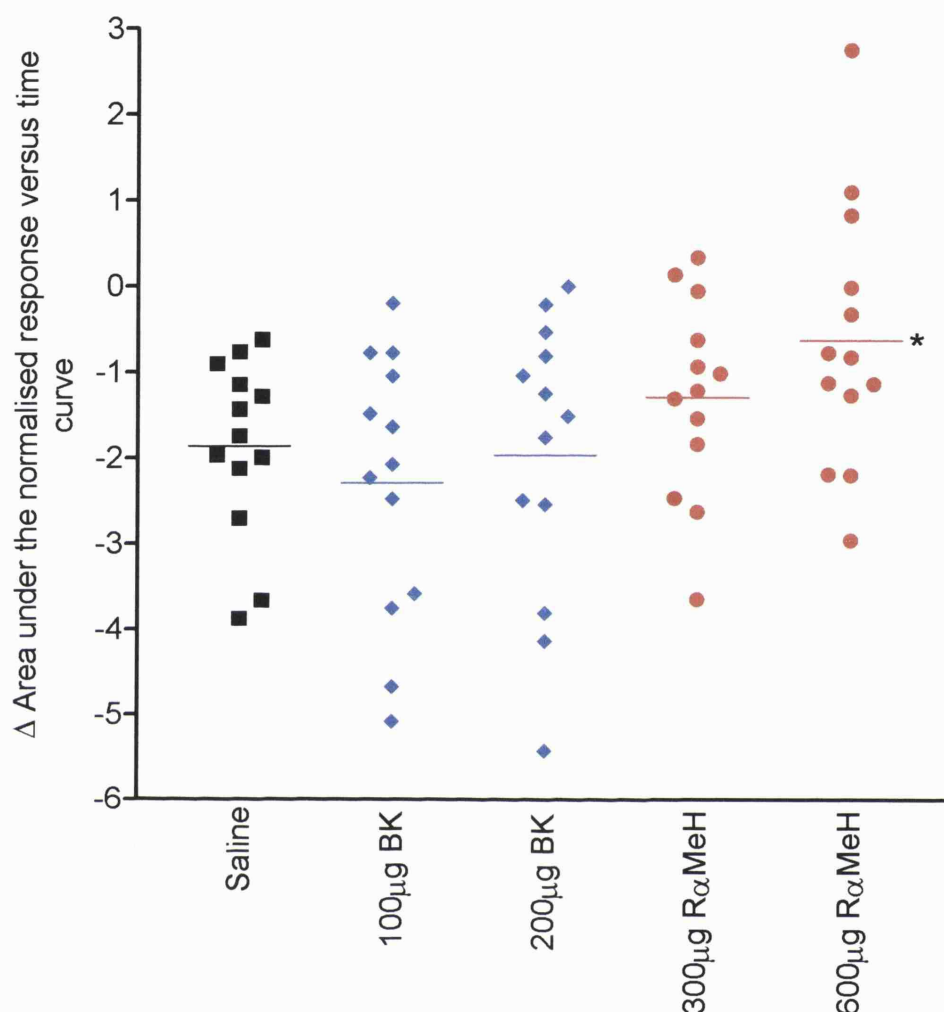


Figure 7.16: The effect of R- $\alpha$ -methylhistamine [R $\alpha$ MeH] or bradykinin [BK] on the nasal blockage caused by corynanthine, 2mg, given as an aerosol into each nostril. Both R- $\alpha$ -methylhistamine [●], 300 $\mu$ g and 600 $\mu$ g, and bradykinin [◆], 100 $\mu$ g and 200 $\mu$ g, were administered as aerosols into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.797cm<sup>2</sup>, s.e.mean of 0.024cm<sup>2</sup>). The data points are the individual responses from 13 subjects and represent the difference between nasal challenge with or without corynanthine in area under the normalised Amin. versus time curve measured over a 15 minute period following the nasal challenge. Horizontal bars represent the mean. \* Significant reduction in nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

caused by R- $\alpha$ -methylhistamine, 600 $\mu$ g ( $p>0.05$ , Wilcoxon sign-rank test), as shown in Figure 7.17.

### **7.3.5 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on nasal patency**

Application, by aerosol into each nostril, of thioperamide, 700 $\mu$ g, either 15 or 45 minutes prior to assessment of nasal patency, had no effect on Amin. as compared to saline ( $p>0.05$ , Wilcoxon sign-rank test), as shown in Figure 7.18.

### **7.3.6 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on R- $\alpha$ -methylhistamine-induced nasal blockage**

Application, by aerosol into each nostril, of R- $\alpha$ -methylhistamine, 600 $\mu$ g, caused a significant reduction in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with thioperamide, 700 $\mu$ g, by aerosol into each nostril 45 minutes prior to challenge with R- $\alpha$ -methylhistamine, inhibited the reduction in AUC by R- $\alpha$ -methylhistamine ( $p<0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by R- $\alpha$ -methylhistamine was not abolished ( $p<0.05$ , Wilcoxon sign-rank test) (Figure 7.19).

### **7.3.7 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on histamine-induced nasal blockage**

Application, by aerosol to each nostril, of histamine, 400 $\mu$ g, caused a significant reduction in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with thioperamide, 700 $\mu$ g, by aerosol into each nostril, either 15 or 45 minutes prior to histamine challenge, had no effect on the reduction in AUC by histamine ( $p>0.05$ , Wilcoxon sign-rank test) (Figure 7.20).

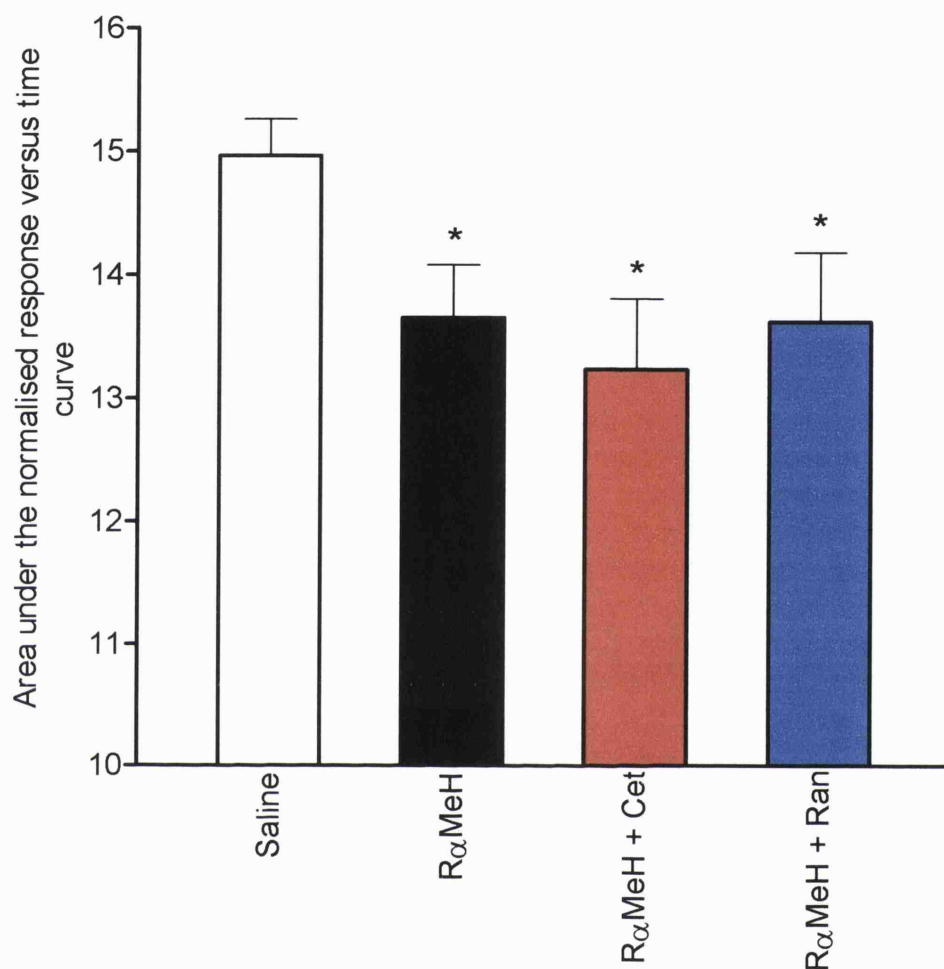


Figure 7.17: The effect of cetirizine [Cet], 20mg, and ranitidine [Ran], 75mg, on the nasal blockage caused by R- $\alpha$ -methylhistamine [R $\alpha$ MeH], 600 $\mu$ g, given as an aerosol into each nostril. Both drugs were given orally 2 hours prior to R- $\alpha$ -methylhistamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.543cm<sup>2</sup>, s.e.mean of 0.021cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of R- $\alpha$ -methylhistamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test).

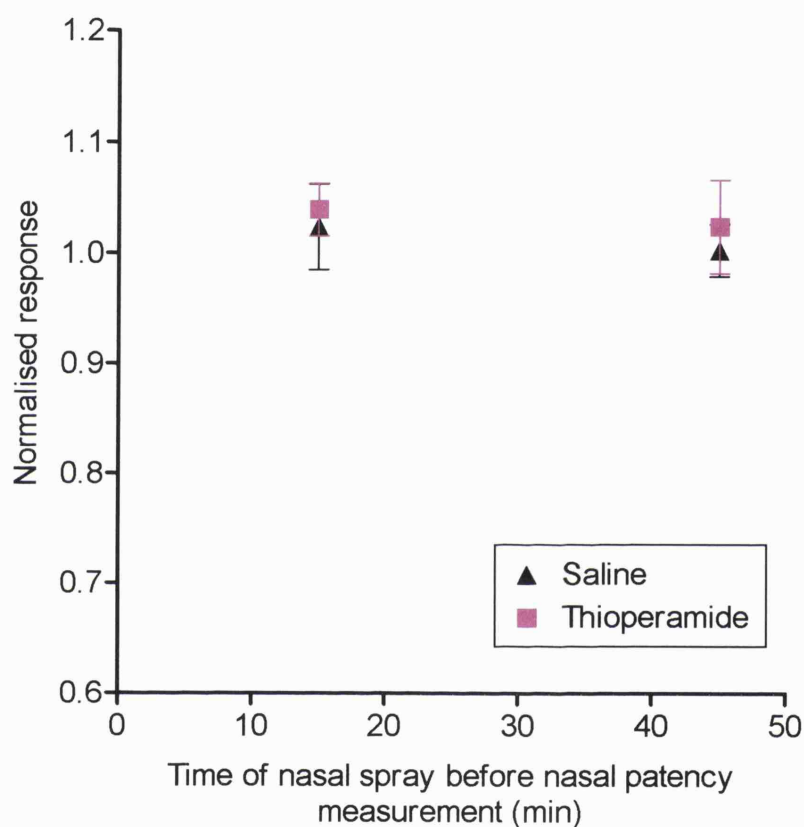


Figure 7.18: The effect of thioperamide, 700 $\mu$ g, administered as an aerosol into each nostril, on the normalized Amin. Amin. was measured immediately before, and then 15 or 45 minutes after each nasal challenge. Amin. values were normalised to the pre-challenge value (mean of 0.657cm<sup>2</sup>, s.e.mean of 0.033cm<sup>2</sup>). Data are means from 8 people. Vertical bars represent s.e.mean.



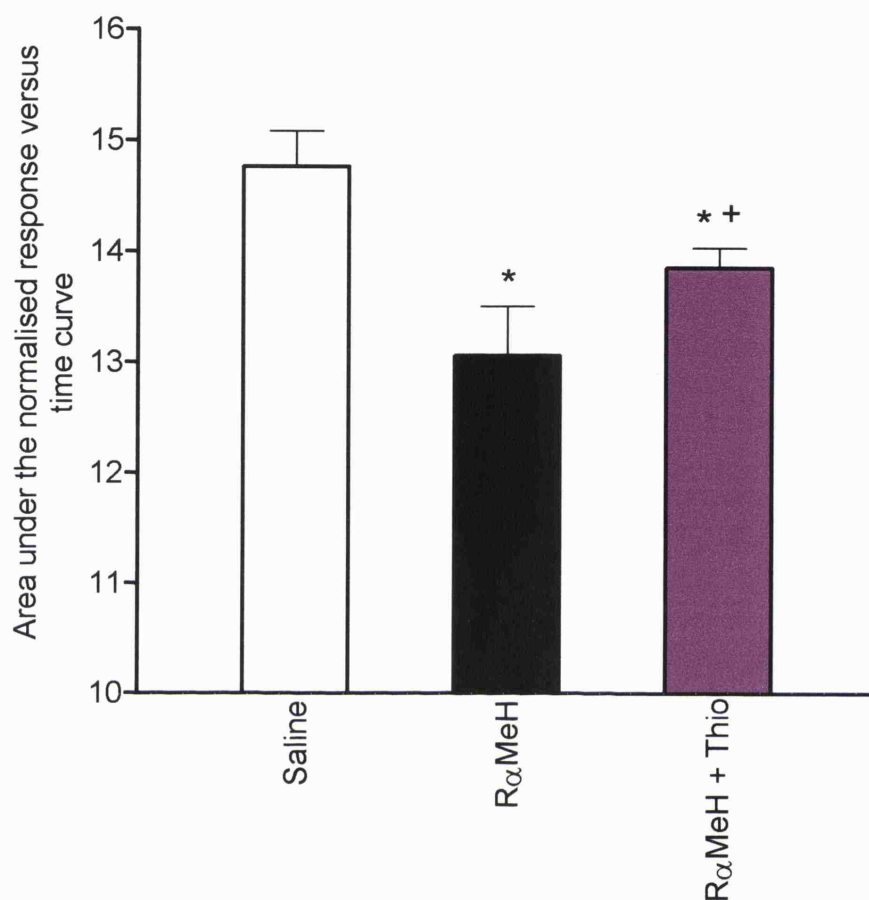


Figure 7.19: The effect of thioperamide [Thio], 700 $\mu$ g, administered as an aerosol into each nostril 45 minutes prior to challenge with R- $\alpha$ -methylhistamine, on the nasal blockage caused by R- $\alpha$ -methylhistamine [R $\alpha$ MeH], 600 $\mu$ g, given as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each R- $\alpha$ -methylhistamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.609cm<sup>2</sup>, s.e.mean of 0.049cm<sup>2</sup>). The data are the means from 10 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of R- $\alpha$ -methylhistamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to R- $\alpha$ -methylhistamine without thioperamide pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

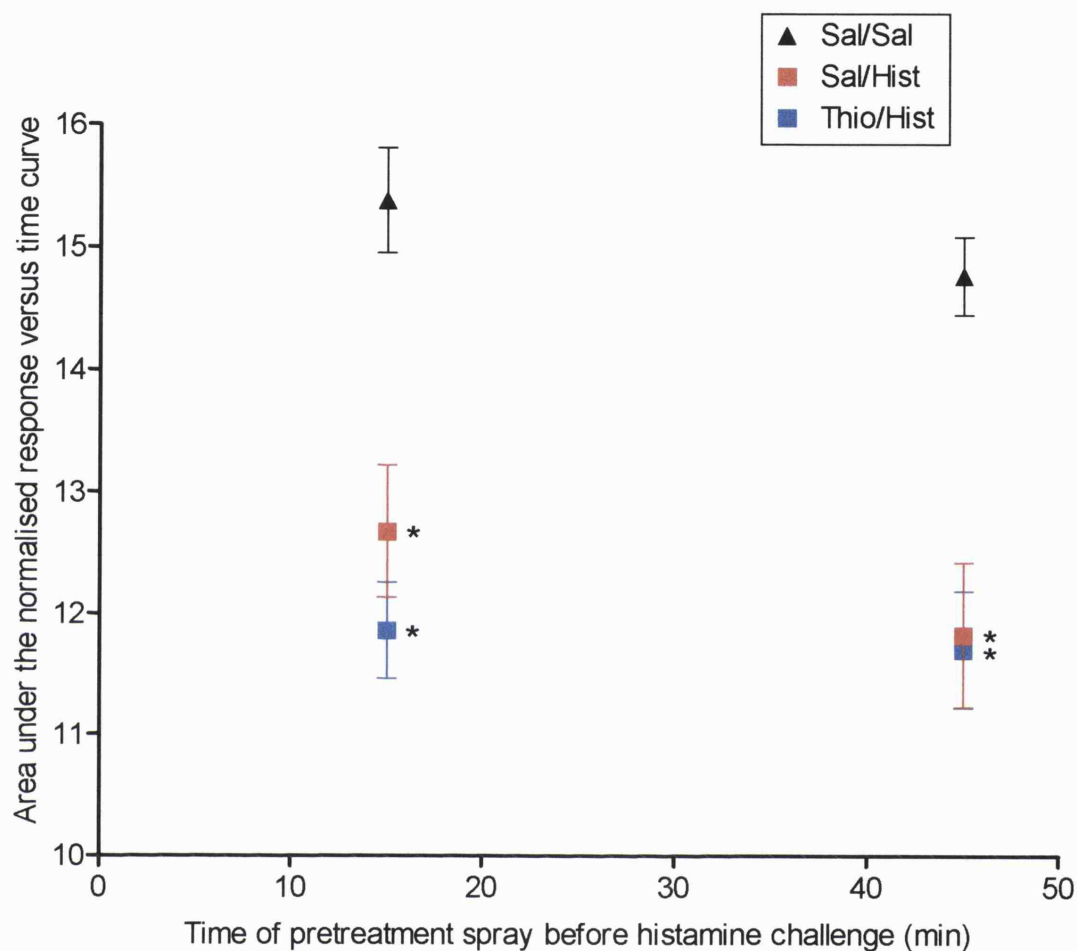


Figure 7.20: The effect of thioperamide [Thio], 700 $\mu$ g, administered as an aerosol into each nostril 15 or 45 minutes prior to challenge with histamine, on the nasal blockage caused by histamine [Hist], 400 $\mu$ g, given as an aerosol into each nostril. Amin. was measured immediately before, and 5, 10 and 15 minutes after each histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.656cm<sup>2</sup>, s.e.mean of 0.032cm<sup>2</sup>). The data are the means from 8 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control [Sal/Sal] ( $p < 0.05$ , Wilcoxon sign-rank test).

### **7.3.8 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, on the reduction of histamine-induced nasal blockage by oral histamine H<sub>1</sub> antagonist, cetirizine**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral cetirizine, 20mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine, 400µg ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test). Pretreatment with a combination of oral cetirizine, 20mg, 2 hours prior to histamine challenge, and thioperamide, 700µg, by aerosol to each nostril, 45 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine pretreated with cetirizine alone ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished by the combined pretreatments ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 7.21).

### **7.3.9 The effect of oral histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination with (i) topical histamine H<sub>3</sub> antagonist, thioperamide, (ii) oral histamine H<sub>2</sub> antagonist, ranitidine, or (iii) both thioperamide and ranitidine, on histamine-induced nasal blockage**

Application, by aerosol to each nostril, of histamine, 400µg, caused a significant reduction in AUC as compared to saline ( $p < 0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral cetirizine, 20mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine ( $p < 0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p < 0.05$ , Wilcoxon sign-rank test) (Figure 7.22).

Pretreatment with a combination of oral cetirizine, 20mg, 2 hours prior to histamine challenge, and thioperamide, 700µg, by aerosol to each nostril, 45 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatments ( $p < 0.05$ , Wilcoxon sign-rank test),

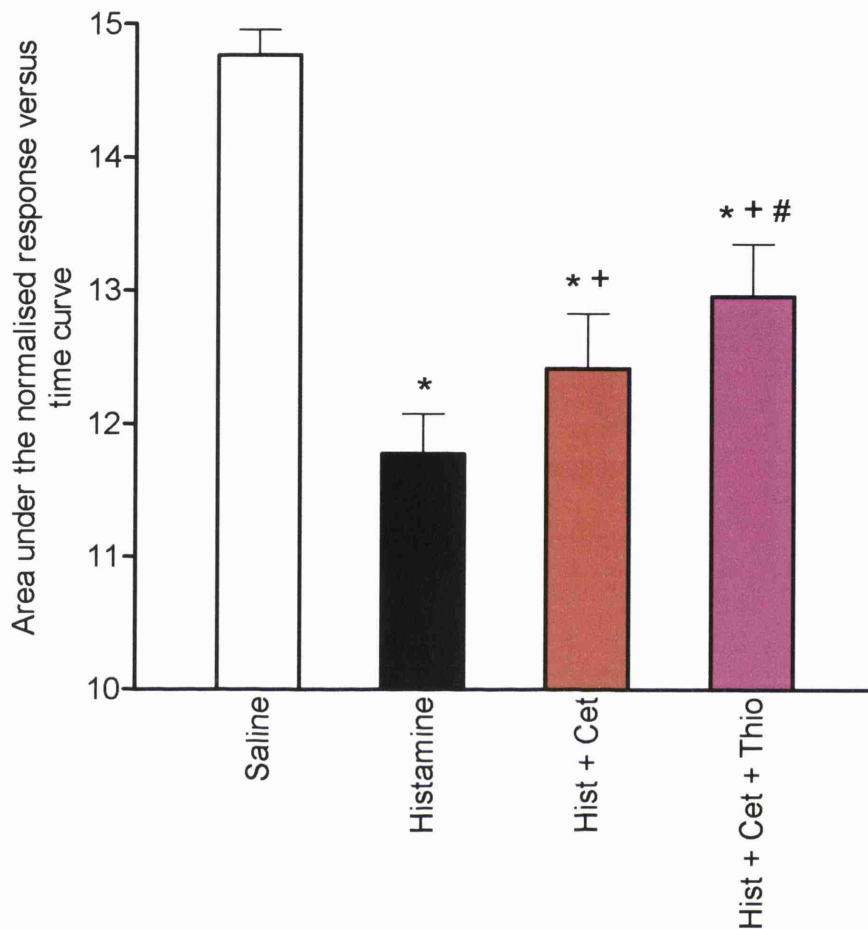


Figure 7.21: The effect of thioperamide [Thio], on the nasal blockage caused by histamine [Hist], 400µg, given as an aerosol into each nostril, in the presence of cetirizine [Cet]. Cetirizine, 20mg, was given orally 2 hours prior to histamine challenge. Thioperamide, 700µg, was administered as an aerosol into each nostril 45 minutes prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.696cm<sup>2</sup>, s.e.mean of 0.045cm<sup>2</sup>). The data are the means from 15 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in the nasal blockage caused by histamine with thioperamide and cetirizine pretreatment as compared to histamine with cetirizine pretreatment alone ( $p < 0.05$ , Wilcoxon sign-rank test).

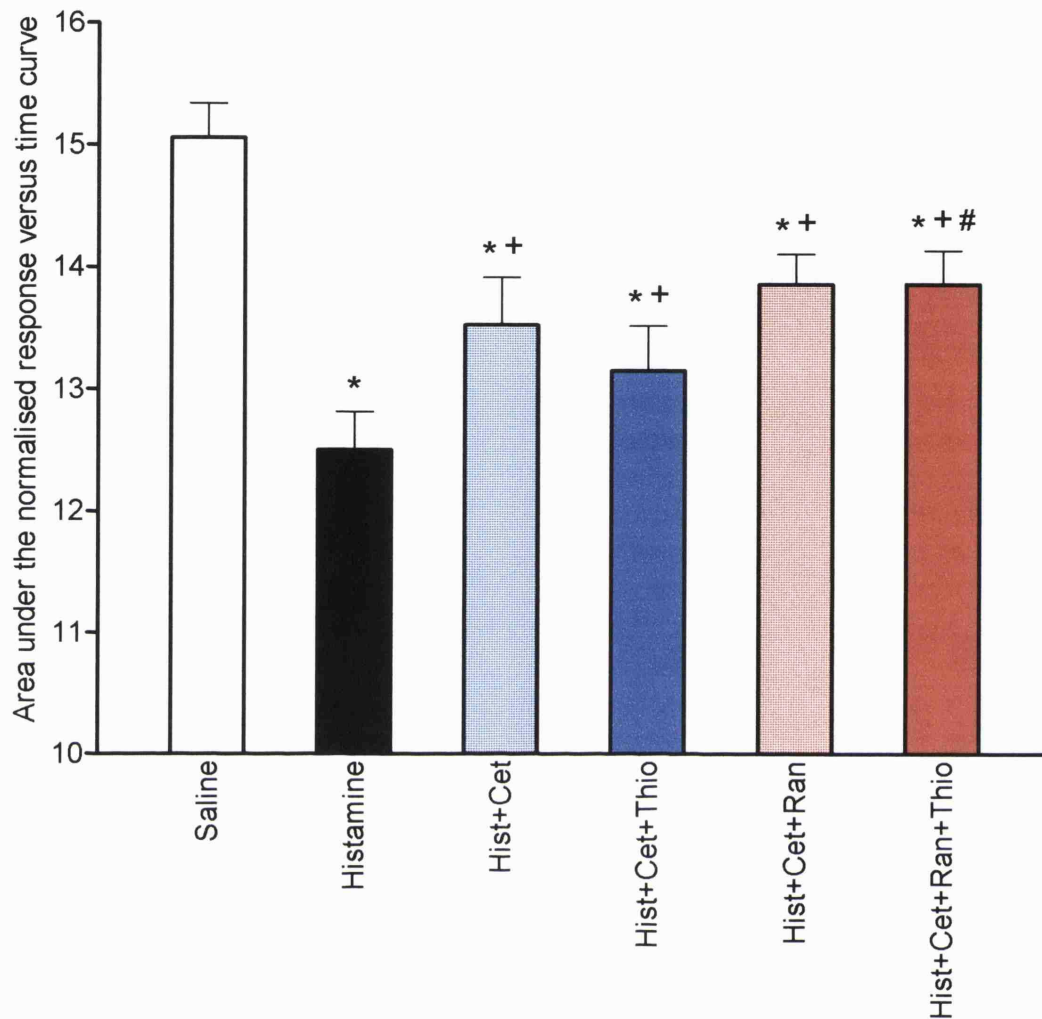


Figure 7.22: The effect of cetirizine [Cet], alone or in combination with either (i) thioperamide [Thio], (ii) ranitidine [Ran] or (iii) both thioperamide and ranitidine, on the nasal blockage caused by histamine [Hist], 400µg, administered as an aerosol into each nostril. Both cetirizine, 20mg, and ranitidine, 150mg, were given orally 2 hours prior to histamine challenge. Thioperamide, 700µg, was administered as an aerosol into each nostril 45 minutes prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.753cm<sup>2</sup>, s.e.mean of 0.029cm<sup>2</sup>). The data are the means from 13 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage with cetirizine pretreatment as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in nasal blockage with ranitidine pretreatment as compared to histamine without ranitidine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

although combined cetirizine and thioperamide pretreatment had no effect on AUC by histamine as compared to histamine with cetirizine alone ( $p>0.05$ , Wilcoxon sign-rank test).

Pretreatment with a combination of oral cetirizine, 20mg, and oral ranitidine, 150mg, both given 2 hours prior to histamine challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatments ( $p<0.05$ , Wilcoxon sign-rank test), although combined cetirizine and ranitidine pretreatment had no effect on AUC by histamine as compared to histamine with cetirizine alone ( $p>0.05$ , Wilcoxon sign-rank test).

Pretreatment with a combination of oral cetirizine, 20mg, oral ranitidine, 150mg, both given 2 hours prior to histamine challenge, and thioperamide, 700 $\mu$ g, by aerosol to each nostril, 45 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatments, and as compared to histamine with a combination of cetirizine and thioperamide ( $p<0.05$ , Wilcoxon sign-rank test). However, combined cetirizine, ranitidine and thioperamide pretreatment had no effect on AUC caused by histamine as compared to histamine with combined cetirizine and ranitidine pretreatment ( $p>0.05$ , Wilcoxon sign-rank test).

None of the pretreatment combinations fully abolished the reduction in AUC by histamine ( $p<0.05$ , Wilcoxon sign-rank test).

#### **7.3.10 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, and oral histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination, on histamine-induced nasal blockage**

Application, by aerosol to each nostril, of histamine, 400 $\mu$ g, caused a significant reduction in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral cetirizine, 20mg, 2 hours prior to nasal challenge, significantly inhibited the reduction in AUC by histamine ( $p<0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished ( $p<0.05$ , Wilcoxon sign-rank test). Pretreatment with thioperamide,



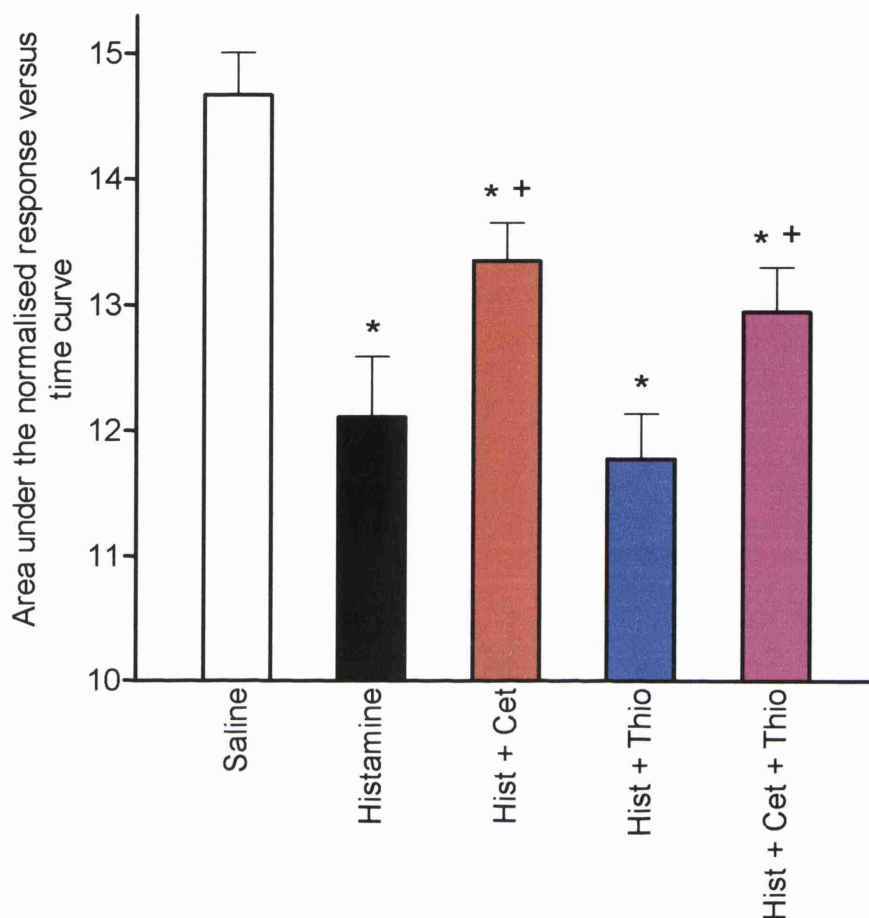


Figure 7.23: The effect of cetirizine [Cet] and thioperamide [Thio] alone and in combination, on the nasal blockage caused by histamine [Hist], 400 $\mu$ g, administered as an aerosol into each nostril. Cetirizine, 20mg, was given orally 2 hours prior to histamine challenge. Thioperamide, 700 $\mu$ g, was administered as an aerosol into each nostril 20, 40 and 60 minutes prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.789cm<sup>2</sup>, s.e.mean of 0.050cm<sup>2</sup>). The data are the means from 9 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without cetirizine pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test).

700µg, by aerosol into each nostril, 20, 40 and 60 minutes prior to histamine challenge, had no effect on the reduction in AUC by histamine ( $p>0.05$ , Wilcoxon sign-rank test) (Figure 7.23).

Pretreatment with a combination of oral cetirizine, 20mg, 2 hours prior to histamine challenge, and thioperamide, 700µg, by aerosol to each nostril, 20, 40 and 60 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine as compared to histamine without pretreatment ( $p<0.05$ , Wilcoxon sign-rank test), although the reduction in AUC by histamine was not abolished by the combined pretreatments ( $p<0.05$ , Wilcoxon sign-rank test). Combined cetirizine and thioperamide pretreatment had no effect on AUC by histamine as compared to histamine with cetirizine alone ( $p>0.05$ , Wilcoxon sign-rank test) (Figure 7.23).

#### **7.3.11 The effect of topical histamine H<sub>3</sub> antagonist, thioperamide, and oral histamine H<sub>1</sub> antagonist, cetirizine, alone or in combination, on the nasal blockage caused by a high dose of histamine.**

Application, by aerosol to each nostril, of histamine, 1000µg, caused a significant reduction in AUC as compared to saline ( $p<0.05$ , Wilcoxon sign-rank test), corresponding to increased nasal blockage measured over the 15 minutes after nasal challenge. Pretreatment with oral cetirizine, 20mg, 2 hours prior to nasal challenge, had no effect on the reduction in AUC by histamine, 1000µg ( $p>0.05$ , Wilcoxon sign-rank test). Pretreatment with thioperamide, 700µg, by aerosol into each nostril, 20, 40 and 60 minutes prior to histamine challenge, had no effect on the reduction in AUC by histamine, 1000µg ( $p>0.05$ , Wilcoxon sign-rank test).

Pretreatment with a combination of oral cetirizine, 20mg, 2 hours prior to histamine challenge, and thioperamide, 700µg, by aerosol to each nostril, 20, 40 and 60 minutes prior to histamine challenge, significantly inhibited the reduction in AUC by histamine, 1000µg, as compared with histamine without pretreatment, histamine with pretreatment of cetirizine alone and histamine with pretreatment of thioperamide alone ( $p<0.05$ , Wilcoxon sign-rank test) (Figure 7.24). The reduction in AUC by histamine was not abolished by the combined pretreatments ( $p<0.05$ , Wilcoxon sign-rank test).



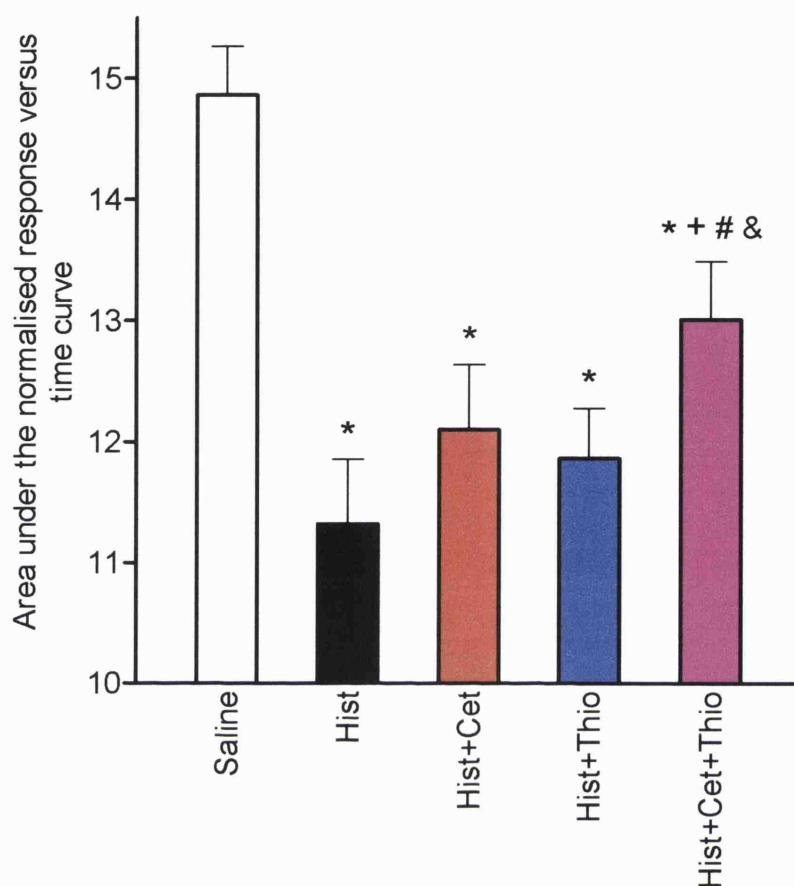


Figure 7.24: The effect of cetirizine [Cet] and thioperamide [Thio] alone and in combination, on the nasal blockage caused by histamine [Hist], 1000 $\mu$ g, administered as an aerosol into each nostril. Cetirizine, 20mg, was given orally 2 hours prior to histamine challenge. Thioperamide, 700 $\mu$ g, was administered as an aerosol into each nostril 20, 40 and 60 minutes prior to histamine challenge. Amin. was measured immediately before, and 5, 10 and 15 minutes after each histamine challenge. Amin. values were normalised to the pre-challenge value (mean of 0.722cm<sup>2</sup>, s.e.mean of 0.037cm<sup>2</sup>). The data are the means from 10 subjects and represent the area under the normalised Amin. versus time curve measured over a 15 minute period following the administration of histamine. Vertical bars represent s.e.mean. \* Significant nasal blockage as compared to saline control ( $p < 0.05$ , Wilcoxon sign-rank test). + Significant reduction in nasal blockage as compared to histamine without antagonist pretreatment ( $p < 0.05$ , Wilcoxon sign-rank test). # Significant reduction in nasal blockage following pretreatment with cetirizine and thioperamide as compared to pretreatment with cetirizine alone ( $p < 0.05$ , Wilcoxon sign-rank test). & Significant reduction in nasal blockage following pretreatment with cetirizine and thioperamide as compared to pretreatment with thioperamide alone ( $p < 0.05$ , Wilcoxon sign-rank test).

## 7.4 Discussion

R- $\alpha$ -methylhistamine, following bilateral nasal administration as an aerosol, 300 $\mu$ g and 600 $\mu$ g, caused a significant reduction in the area under the normalised Amin. versus time curve measured over a period of 15 minutes (AUC). This reduction in AUC represents an objectively measured reduction in nasal patency or, alternatively, increased nasal blockage. The stereoisomer of R- $\alpha$ -methylhistamine, S- $\alpha$ -methylhistamine, however, following bilateral nasal administration as an aerosol, 600 $\mu$ g, failed to cause any significant nasal blockage. Although both are full agonists at H<sub>3</sub> receptors, S- $\alpha$ -methylhistamine has a binding affinity a hundred and twenty times less than its stereoisomer, R- $\alpha$ -methylhistamine (Arrang JM *et al.*, 1987). The stereo-specific nasal blockage caused by R- $\alpha$ -methylhistamine suggests that the nasal response is not due to any chemical toxicity of the substance but rather due to the activation of H<sub>3</sub> receptors. This hypothesis is supported by the observation that R- $\alpha$ -methylhistamine-induced nasal blockage is reduced by pretreatment with thioperamide, the H<sub>3</sub> antagonist, but not by pretreatment with cetirizine, the H<sub>1</sub> antagonist, or ranitidine, the H<sub>2</sub> antagonist. That R- $\alpha$ -methylhistamine activates H<sub>3</sub> receptors, but not H<sub>1</sub> or H<sub>2</sub> receptors is in agreement with the published affinity of R- $\alpha$ -methylhistamine for these receptors (Arrang JM *et al.*, 1987). However, the present data conflicts with the only other *in vivo* study of R- $\alpha$ -methylhistamine in humans (Kavanagh GM *et al.*, 1998). In this study, R- $\alpha$ -methylhistamine caused wheal and flare responses in human skin following intradermal administration which was not reduced by the single dose of thioperamide that was used, but was slightly inhibited by oral terfenadine, an H<sub>1</sub> antagonist.

Both R- $\alpha$ -methylhistamine and thioperamide have some activity at human H<sub>4</sub> receptors (Nakamura T *et al.*, 2000; Zhu Y *et al.*, 2001) and it is possible that the R- $\alpha$ -methylhistamine-induced nasal blockage is mediated via H<sub>4</sub> receptors (or a combination of H<sub>3</sub> and H<sub>4</sub> receptors). High levels of H<sub>3</sub> receptor mRNA have been found in human nasal mucosa (Varty LM *et al.*, 2004). Unfortunately, there have been no studies of nasal H<sub>4</sub> receptors. The published evidence suggests that H<sub>4</sub> receptors are primarily found on leukocytes (Repka-Ramirez MS, 2003). Although there is no conclusive evidence to discount the H<sub>4</sub> receptor in mediating some or all of the R- $\alpha$ -methylhistamine-induced nasal blockage, the response to R- $\alpha$ -

methylhistamine in an *in vitro* preparation of human nasal mucosa was antagonized by clobenpropit (Varty LM *et al.*, 2004). Clobenpropit is an antagonist at human H<sub>3</sub> receptors but is an agonist at human H<sub>4</sub> receptors (Buckland *et al.*, 2003). As clobenpropit, in this published study, had no activity on its own, and R- $\alpha$ -methylhistamine was inhibited by clobenpropit, it suggests that it is indeed the H<sub>3</sub> receptor that mediates the nasal response to R- $\alpha$ -methylhistamine in humans.

The experiment investigating the effect of corynanthine on R- $\alpha$ -methylhistamine- and bradykinin-induced nasal blockage (Chapters 7.2.3 and 7.3.3 for protocol and results, respectively) was designed to reveal the mechanism by which the activation of H<sub>3</sub> receptors causes nasal blockage. The majority of studies showing a vasodilatory effect of H<sub>3</sub> agonists have indicated that this vasodilation is mediated via an indirect mechanism: the inhibition of sympathetic neuronal activity (Beyak M *et al.*, 1995; Valentine AF *et al.*, 1999; Varty L *et al.*, 2002; Varty LM *et al.*, 2004); although some studies have demonstrated direct H<sub>3</sub>-mediated vasodilation via vascular H<sub>3</sub> receptors (Ea Kim L *et al.*, 1992; Champion HC *et al.*, 1998). For a presynaptic H<sub>3</sub> receptor on sympathetic neurones to mediate nasal vasodilation by inhibiting synaptic activity, basal release of noradrenaline (NA) must first be established. As demonstrated in Chapter 6, the sympathetic nervous system does, in fact, play an active role in the control of nasal patency. NA, released from nasal sympathetic neurones, binds to postsynaptic  $\alpha_1$ -adrenoceptors, and causes vasoconstriction. Inhibition of  $\alpha_1$ -adrenoceptors by corynanthine was shown to cause nasal blockage, presumably by removing the decongestant effect of the endogenous NA. Thus, it is possible that H<sub>3</sub> receptor activation could cause nasal blockage by inhibiting NA release.

As shown in Figure 7.15, corynanthine, 2mg, caused significant nasal blockage, in agreement with the data in Chapter 6 and again demonstrating that basally-released NA actively controls nasal patency. Both R- $\alpha$ -methylhistamine, 600 $\mu$ g, and bradykinin, 100 and 200 $\mu$ g, also caused nasal blockage. Bradykinin has previously been shown to cause nasal blockage at these doses (Austin CE *et al.*, 1994a), via activation of endothelial bradykinin B<sub>2</sub> receptors (Dear JW *et al.*, 1996a; Dear JW *et al.*, 1996b; Honing ML *et al.*, 2000). Curiously, R- $\alpha$ -methylhistamine, 300 $\mu$ g, failed to cause significant nasal blockage – a result that conflicts with previous data (Figure 7.13), possibly indicating a variability of nasal responses at the foot of the

dose-response curve. Bradykinin and corynanthine, when given in combination, had an additive effect on nasal blockage, causing greater nasal blockage than either compound alone. However, a combination of corynanthine, 2mg, and R- $\alpha$ -methylhistamine, 600 $\mu$ g, did not produce significantly more nasal blockage than either compound alone. Given that R- $\alpha$ -methylhistamine, 300 $\mu$ g, failed to cause significant nasal blockage with these subjects, it is unsurprising that the combined nasal challenge of corynanthine, 2mg, and R- $\alpha$ -methylhistamine, 300 $\mu$ g, caused a significant increase in nasal blockage as compared to R- $\alpha$ -methylhistamine, 300 $\mu$ g, alone but not compared to corynanthine, 2mg, alone.

The data in Figure 7.15 suggests that the nasal response to corynanthine is altered by its co-administration with R- $\alpha$ -methylhistamine, but not with bradykinin. The data was re-analysed to show the effect of R- $\alpha$ -methylhistamine and bradykinin on the nasal response to corynanthine, 2mg (Figure 7.16). In this figure, the difference in AUC between nasal challenge with or without corynanthine ( $\Delta$ AUC) for saline, R- $\alpha$ -methylhistamine, 300 $\mu$ g and 600 $\mu$ g, and bradykinin, 100 $\mu$ g and 200 $\mu$ g, is shown. Co-administration of bradykinin has no effect on the  $\Delta$ AUC caused by corynanthine as compared to saline. The data suggests that the mechanisms by which bradykinin and corynanthine cause nasal blockage do not interact, presumably as bradykinin only causes nasal blockage via endothelial-dependent vasodilation and corynanthine only causes nasal blockage by inhibiting the activation of  $\alpha_1$ -adrenoceptors by endogenous NA. This lack of interaction between bradykinin and sympathetic activity conflicts with some reports in the literature that describe a presynaptic mechanism by which bradykinin promotes the release of NA (Llona I *et al.*, 1991; Basbaum AI *et al.*, 1991). However, in support of the present data, there are no reports of bradykinin augmenting sympathetic activity in the nasal mucosa. In contrast with bradykinin, co-administration of R- $\alpha$ -methylhistamine significantly reduced the effect of corynanthine on  $\Delta$ AUC as compared to saline, indicating that corynanthine causes less nasal blockage following H<sub>3</sub> receptor activation. This suggests that the mechanisms by which R- $\alpha$ -methylhistamine and corynanthine cause nasal blockage interact. As R- $\alpha$ -methylhistamine has no affinity for  $\alpha_1$ -adrenoceptors (Varty L *et al.*, 2002) it seems reasonable to suggest that R- $\alpha$ -methylhistamine causes nasal blockage by inhibiting the NA- $\alpha_1$ -adrenoceptor pathway upstream of the receptor. In this way, H<sub>3</sub> receptor activation would decrease the concentration of endogenous NA at vascular adrenoceptors, thus

reducing the effect of  $\alpha_1$ -adrenoceptor antagonism by corynanthine. The possible mechanisms by which R- $\alpha$ -methylhistamine could reduce the synaptic NA concentration are: inhibition of NA synthesis, inhibition of NA release, augmentation of NA uptake and augmentation of NA metabolism. There are no reports of R- $\alpha$ -methylhistamine affecting NA synthesis. There is only one report in the literature investigating the effect of R- $\alpha$ -methylhistamine on catecholamine uptake, and in this study, none of the histamine receptor agonists used altered serotonin uptake in rat blood platelets (Pawlak D *et al.*, 1996). R- $\alpha$ -methylhistamine has been shown to inhibit weakly monoamine oxidase in human brains (Sakurai E *et al.*, 2001). However, this is more likely to lead to an increase in NA in the synapse, not a decrease, and so it is doubtful that this mechanism explains the data in Figure 7.13. The majority of the evidence in the literature points to inhibition of NA release as the mechanism by which activation of H<sub>3</sub> receptors reduces synaptic NA levels (Ishikawa S *et al.*, 1987; Molderings GJ *et al.*, 1992; Valentine AF *et al.*, 1999; Yamasaki T *et al.*, 2001; Silver RB *et al.*, 2002).

In conclusion, it appears that R- $\alpha$ -methylhistamine causes nasal blockage in normal, non-atopic individuals via activation of inhibitory presynaptic thioperamide-sensitive H<sub>3</sub> receptors on sympathetic nasal neurones, thereby reducing the levels of NA at vascular  $\alpha$ -adrenoceptors. This is in agreement with Varty LM *et al.* (2004), who reported that R- $\alpha$ -methylhistamine inhibited *in vitro* electric field stimulation-induced vasoconstriction of human nasal mucosa, an effect that was sensitive to the H<sub>3</sub> antagonist, clobenpropit. This mechanism has also been demonstrated in pig nasal mucosa, where, in addition to showing thioperamide- and clobenpropit-sensitive R- $\alpha$ -methylhistamine inhibition of electric field stimulation-induced vasoconstriction, R- $\alpha$ -methylhistamine failed to inhibit the contractions caused by exogenous NA (Varty L *et al.*, 2002). The R- $\alpha$ -methylhistamine experiments suggested that activation of nasal H<sub>3</sub> receptors could cause nasal blockage, but whether or not the H<sub>3</sub> receptor contributed to histamine-induced nasal blockage was not certain. This was investigated using the H<sub>3</sub> antagonist, thioperamide.

Thioperamide, following bilateral nasal administration as an aerosol, 700 $\mu$ g, failed to affect the Amin. recorded 15 or 45 minutes following nasal challenge. This observation was not unexpected as administration of H<sub>1</sub> and H<sub>2</sub> antagonists also has no effect on resting nasal patency as shown in Figures 3.11, 4.17 and 5.4,

indicating that, in the unchallenged nasal mucosa, histamine is not present in significant levels.

Thioperamide, 700µg, administered either 15 or 45 minutes prior to histamine challenge, had no effect on the nasal blockage caused by histamine, 400µg (Figure 7.20). In a cat model of allergic rhinitis, McLeod *et al.* (1999) demonstrated that the nasal blockage caused by compound 48/80 was not significantly inhibited by either an H<sub>1</sub> antagonist alone or an H<sub>3</sub> antagonist alone, but was significantly reduced by a combination of H<sub>1</sub> and H<sub>3</sub> antagonism. In Figure 7.21, a combination of cetirizine, 20mg, administered 2 hours prior to histamine challenge, and thioperamide, 700µg, given 45 minutes prior to histamine challenge, significantly reduced the nasal blockage caused by histamine, 400µg. This observation suggested that H<sub>3</sub> receptors mediated some of the histamine-induced nasal blockage. Interestingly, as also shown in Chapter 3, pretreatment with cetirizine, the H<sub>1</sub> antagonist, alone significantly reduced the histamine-induced nasal blockage, which conflicts with the cat data published by McLeod *et al.* (1999).

If the nasal blockage caused by histamine, 400µg, is sensitive to H<sub>3</sub> antagonism, it may be possible to abolish the nasal blockage caused by histamine, 400µg, using a combination of H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> antagonism. However, as shown in Figure 7.22, not only did the combination of cetirizine, ranitidine and thioperamide fail to abolish the nasal blockage caused by histamine, 400µg; but in addition, thioperamide, 700µg, given 45 minutes prior to histamine challenge, when co-administered with either cetirizine or a combination of cetirizine and ranitidine, failed to further reduce the histamine-induced nasal blockage. The data shown in Figure 7.22 clearly conflicts with the data from the previous study (Figure 7.21). One possibility for the inconsistent results could have been the time-course of the thioperamide pretreatment. The only *in vivo* study of the effect of thioperamide on nasal responses used oral and intravenous administration, not topical administration, with pretreatment 2 hours and 10 minutes prior to nasal challenge, respectively (McLeod RL *et al.*, 1999b). In an *in vitro* study of porcine nasal mucosa, thioperamide was allowed to equilibrate with the tissue for 1 hour prior to experimentation (Varty L *et al.*, 2002). Perhaps, the thioperamide in the present studies was subject to removal from the tissue, either via metabolism or venous clearance. There are no reports of thioperamide pharmacokinetics in humans, although its half-life in rats is

approximately 26 minutes (Sakurai E *et al.*, 1994). To counteract this possible physiological reduction of thioperamide after administration, the protocol was adapted so that thioperamide, 700µg, was administered as an aerosol into each nostril 20, 40 and 60 minutes prior to nasal challenge.

The effect of repeated topical thioperamide on the nasal blockage caused by histamine, 400µg, is shown in Figure 7.23. Thioperamide, either alone, or in combination with cetirizine, again had no significant effect on histamine-induced nasal blockage. The data suggest that, although R-α-methylhistamine causes thioperamide-sensitive nasal blockage, the nasal blockage caused by histamine, 400µg, is unaffected by the H<sub>3</sub> antagonist at this dose. Although both R-α-methylhistamine and histamine are full agonists at H<sub>3</sub> receptors, R-α-methylhistamine has a binding affinity 15 times greater than histamine. Assuming equal distribution throughout the nasal mucosa following administration as an aerosol into each nostril, and given their similar relative molecular weights, it seems reasonable to estimate that histamine, 400µg, and R-α-methylhistamine, 600µg, are present at similar concentrations at the nasal H<sub>3</sub> receptors. However, due to the relatively weaker binding affinity of histamine, its activation of H<sub>3</sub> receptors would be less than that of R-α-methylhistamine. As the nasal blockage caused by histamine, 400µg, is, although significant, not vast, it is possible that the H<sub>3</sub> component is too small for the nasal blockage caused by histamine, 400µg, to be significantly reduced by thioperamide, 700µg. The most practical method to solve this problem was simply to increase the dose of histamine, thereby potentially exposing a greater thioperamide-sensitive H<sub>3</sub> component.

As expected, histamine, 1000µg, caused significant nasal blockage (Figure 7.24). Pretreatment with cetirizine, 20mg (the dose that was shown in Chapter 3 to be maximally effective in reducing the nasal blockage caused by histamine, 400µg), had no significant effect on the nasal blockage caused by histamine, 1000µg – although there does appear to be a trend for cetirizine, 20mg, to inhibit histamine, 1000µg: the p value was 0.08. It is likely that the histamine concentration was too high for significant competition by cetirizine, 20mg. Pretreatment with thioperamide, 700µg, given 20, 40 and 60 minutes prior to histamine challenge, also had no significant effect on the nasal blockage caused by histamine, 1000µg. However, pretreatment with the combination of cetirizine and thioperamide significantly

reduced the nasal blockage caused by histamine, 1000µg. Therefore, by using a higher dose of histamine, the thioperamide-sensitive H<sub>3</sub> component of histamine-induced nasal blockage was exposed. Thus, as demonstrated in this chapter and Chapters 3 and 4, histamine appears to cause nasal blockage in normal, non-atopic humans via H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors.

Interestingly, the data in Figure 7.24 mimics the compound 48/80 data published by McLeod *et al.* (1999): neither H<sub>1</sub> nor H<sub>3</sub> antagonist alone had any effect on the nasal blockage, but a combination was effective. In the McLeod *et al.* (1999) study, a combination of relatively low concentrations of H<sub>1</sub> and H<sub>3</sub> antagonists significantly reduced compound 48/80-induced nasal blockage in the cat, whereas high doses of either antagonist alone had no effect. However, given the H<sub>1</sub> antagonist-sensitivity of nasal blockage caused by histamine, 400µg, in normal human subjects (Figures 3.11, 3.12 and 3.13), it seems reasonable to expect higher doses of cetirizine to inhibit significantly the nasal blockage caused by histamine, 1000µg.

Whether or not pretreatment with thioperamide alone can significantly inhibit histamine-induced nasal blockage is not clear. All the *in vitro* and *in vivo* studies showing H<sub>3</sub>-mediated nasal blockage/vasodilation via inhibition of sympathetic activity were performed in the presence of high doses of H<sub>1</sub> antagonists (McLeod RL *et al.*, 1999b; Varty L *et al.*, 2002; Varty LM *et al.*, 2004). Indeed, the majority of reports of H<sub>3</sub>-induced vasodilation via presynaptic inhibition of sympathetic activity in other tissues either used a selective H<sub>3</sub> agonist or used histamine (or antigen or compound 48/80) in the presence of H<sub>1</sub> antagonists (Ichinose M *et al.*, 1989; Danko G *et al.*, 1994; Beyak M *et al.*, 1995; Valentine AF *et al.*, 1999). Although, in an *in vitro* study of H<sub>3</sub>-mediated vasodilation in human saphenous vein, thioperamide inhibited the presynaptic effects of histamine without the presence of an H<sub>1</sub> antagonist (Molderings GJ *et al.*, 1992). Perhaps thioperamide sensitivity in the human nasal mucosa, and thus the H<sub>3</sub> component, is only revealed after activation of H<sub>1</sub> receptors is inhibited; that H<sub>1</sub> and H<sub>3</sub> receptors somehow interact. As mentioned in Chapter 1.7.3.1, excitatory H<sub>1</sub> receptors have been shown on human nasal sympathetic neurones (Kubo N *et al.*, 1989b). It is possible that histamine activates these presynaptic H<sub>1</sub> receptors, leading to neuronal intracellular pathways that inhibit the H<sub>3</sub>-mediated reduction of synaptic activity, as shown in Figure 7.25.



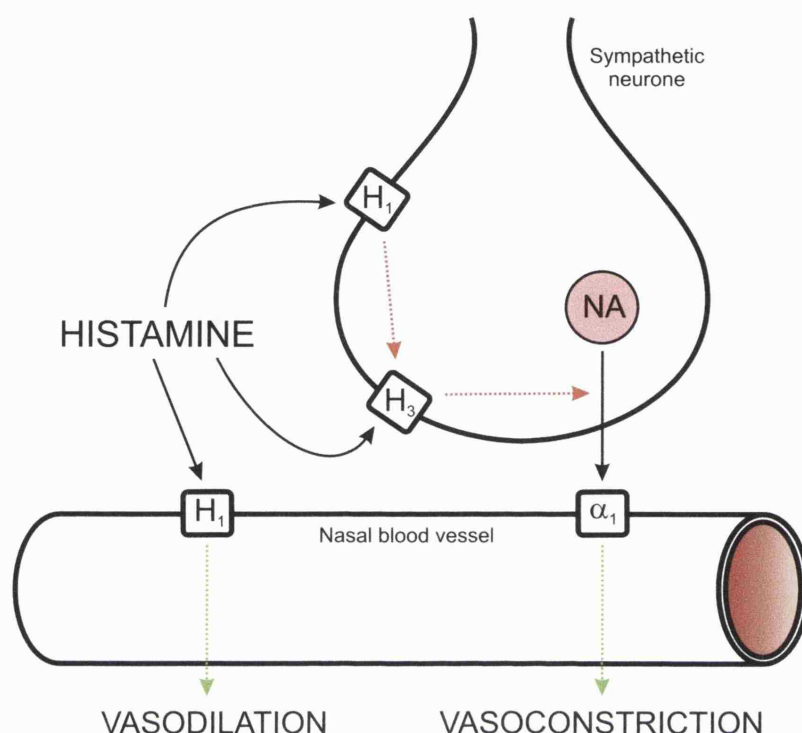


Figure 7.25: Hypothetical role of presynaptic  $H_1$  receptors on nasal sympathetic neurones. NA = noradrenaline,  $\alpha_1$  =  $\alpha_1$ -adrenoceptors. Red arrows denote inhibition, Green arrows denote activation.

Therefore, from this hypothesis, sole activation of  $H_3$  receptors, for example following R- $\alpha$ -methylhistamine challenge, would lead to thioperamide-sensitive nasal blockage (via inhibition of basal NA release). However, following histamine challenge, both  $H_1$  and  $H_3$  receptors would be activated. In this hypothesis, activation of presynaptic  $H_1$  receptors would inhibit any nasal blockage caused by presynaptic  $H_3$  receptors, thus thioperamide, alone, would have no effect. Therefore the nasal blockage caused by exogenous histamine would be a balance of the vasodilation caused by activation of vascular histamine receptors (thought mainly to be  $H_1$  receptors) and the vasoconstriction caused by the basally released NA (which remains unchanged by presynaptic events). Following pretreatment of cetirizine, vascular  $H_1$  receptors would be protected from exogenously administered histamine, thus reducing nasal blockage, but, in addition, cetirizine pretreatment would prevent histamine, via presynaptic  $H_1$  receptors, reducing the influence of the presynaptic  $H_3$  receptors on sympathetic activity. Thus the  $H_3$  component would be exposed and so would explain why thioperamide is only effective in combination with  $H_1$  antagonists.

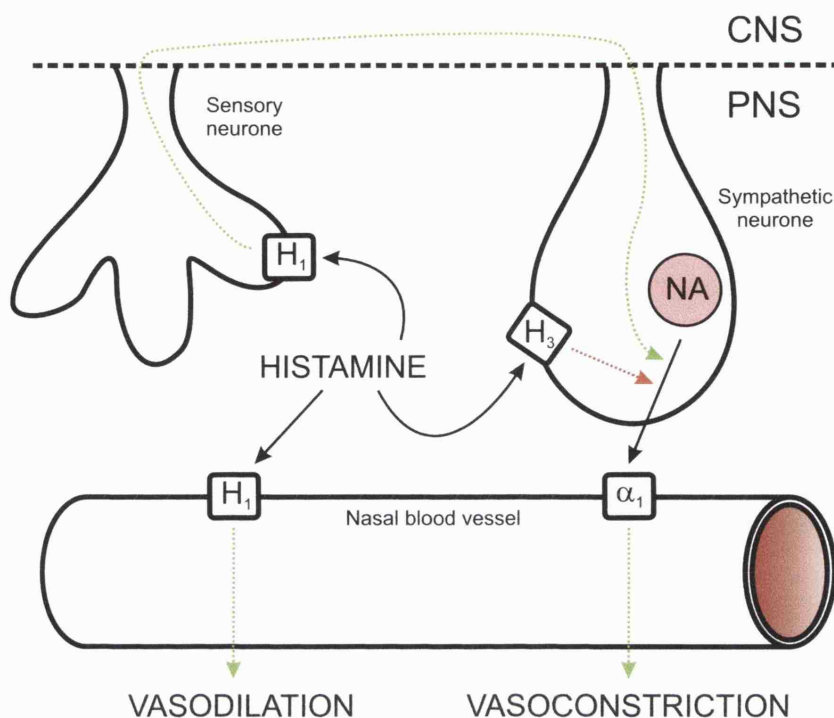


Figure 7.26: Hypothetical role of sensory  $H_1$  receptors on nasal sympathetic neuronal activity. NA = noradrenaline,  $\alpha_1$  =  $\alpha_1$ -adrenoceptors, CNS = central nervous system, PNS = peripheral nervous system. Red arrows denote inhibition, Green arrows denote activation.

Alternately, the data could be explained by a central reflex-induced increase in sympathetic activity mediated by  $H_1$  receptors on nasal sensory neurones, as shown in Figure 7.26. There is substantial evidence to show that activation of sensory neurones leads to an increase in nasal parasympathetic activity via a central reflex (Baraniuk JN, 1997). Indeed, histamine-induced rhinorrhea can be inhibited by muscarinic antagonists (Birchall MA *et al.*, 1996). Histamine is thought to stimulate central reflexes by activating  $H_1$  receptors on sensory neurones (Howarth PH *et al.*, 2000). However, there is little substantive evidence showing central reflex-mediated increases in sympathetic activity (as demonstrated in Figure 3.10), although, as discussed in Chapter 3.4, unilateral histamine challenge has previously been shown to increase contralateral nasal patency (Birchall MA *et al.*, 1993). Nevertheless, in this hypothesis, exogenous histamine would activate both sensory  $H_1$  receptors (increasing sympathetic activity) and sympathetic  $H_3$  receptors (decreasing sympathetic activity), thus there would be no  $H_3$ -mediated nasal blockage to be sensitive to thioperamide. However, following pretreatment of an  $H_1$  antagonist, the central reflex-mediated increase in sympathetic activity would disappear. Thus the activated  $H_3$  receptor would decrease basal NA release,

thereby causing thioperamide-sensitive nasal blockage. Of course, this central reflex would not affect R- $\alpha$ -methylhistamine-induced nasal blockage and so would explain why the nasal response to the H<sub>3</sub> selective agonist was sensitive to thioperamide.

For either of these hypotheses to describe accurately the data demonstrated in this chapter and Chapter 3, the anti-inflammatory effects of neuronal H<sub>1</sub> receptor activation (either on sensory or sympathetic neurones) cannot be so great as to functionally antagonize the pro-inflammatory effects of vascular H<sub>1</sub> receptor activation. Nasal challenge with the H<sub>1</sub> selective agonist, betahistine, has previously been shown to cause nasal blockage (Shelton D *et al.*, 1994), therefore the net gain of H<sub>1</sub> activation is nasal blockage (proinflammatory). In terms of effect on nasal patency in normal, non-atopic individuals, vascular H<sub>1</sub> receptors are far more potent than sympathetic presynaptic H<sub>3</sub> receptors, which are probably of similar potency to these hypothesized anti-inflammatory neuronal H<sub>1</sub> receptors.

These hypotheses could be tested by assaying the levels of NA or 3,4-dihydroxyphenylglycol (DHPG) in lavages following unilateral nasal challenge with betahistine. As discussed in Chapter 6.6, both NA and its metabolite, DHPG, have been recovered from nasal lavages and analysed using electrochemical high pressure liquid chromatography. If activation of presynaptic H<sub>1</sub> receptors increases NA release, unilateral nasal challenge with betahistine would possibly cause a measurable increase in NA/DHPG levels in the ipsilateral nasal cavity only. Whereas, if NA/DHPG levels were to increase in both ipsi- and contralateral nasal cavities, it would suggest that sympathetic activity is increased by a centrally reflex-mediated mechanism.

The failure of thioperamide, 700 $\mu$ g, to reduce histamine-induced nasal blockage without co-administration with an H<sub>1</sub> antagonist, may reflect the inadequacies of thioperamide as a tool for the study of nasal responses instead of demonstrating an anti-inflammatory role of nasal H<sub>1</sub> receptors. Indeed, H<sub>3</sub> antagonists other than thioperamide may be able to reduce histamine-induced nasal blockage without the requirement of co-administration with H<sub>1</sub> antagonists. As shown in Figure 7.19, thioperamide, 700 $\mu$ g, given 45 minutes prior to nasal challenge, did significantly reduce, but failed to abolish, the nasal blockage caused by R- $\alpha$ -methylhistamine,

600µg. Either the very selective (Arrang JM *et al.*, 1987) R-α-methylhistamine causes nasal blockage via non-H<sub>3</sub>-mediated mechanisms or thioperamide, 700µg, given 45 minutes prior to nasal challenge, is insufficient in completely antagonizing R-α-methylhistamine, 600µg. This may be resolved by increasing the thioperamide dose. Perhaps, though, increasing the concentration of thioperamide at nasal H<sub>3</sub> receptors is hindered by poor tissue penetration and rapid clearance. Thioperamide has been shown to distribute well following intravenous administration in the rat (Sakurai E *et al.*, 1994). Although no studies of thioperamide nasal absorption have been published, McLeod *et al.* (1999) only demonstrated effective reduction (when in combination with an H<sub>1</sub> antagonist) of feline nasal blockage caused by compound 48/80, 1% w/v, with relatively high doses of oral thioperamide, 30mg/kg, and intravenous thioperamide, 3mg/kg. It is interesting to note that the McLeod *et al.* (1999) study also used clobenpropit, another H<sub>3</sub> antagonist, and showed significant effects with doses ten times less than thioperamide (note that the relative molecular weight of thioperamide maleate and clobenpropit dihydrobromide is 408g and 470g, respectively). This suggests that clobenpropit may be a better pharmacological tool for the investigation of nasal H<sub>3</sub> receptors. It would be interesting to repeat the high dose histamine challenge study (Chapter 7.2.11 and 7.3.11 for protocol and results, respectively) using clobenpropit to assess whether or not a different H<sub>3</sub> antagonist would be able to reduce histamine-induced nasal blockage alone.

## 7.5 Summary

- 1) R- $\alpha$ -methylhistamine, 300 and 600 $\mu$ g, induced a significant decrease in the patency of the nasal airways of normal individuals, observed as an increase in nasal blockage. S- $\alpha$ -methylhistamine, 600 $\mu$ g, had no significant effect on nasal patency.
- 2) Pretreatment with oral cetirizine, 20mg, or ranitidine, 75mg, had no effect on the nasal blockage caused by R- $\alpha$ -methylhistamine, 600 $\mu$ g.
- 3) Pretreatment with topical thioperamide, 700 $\mu$ g, significantly reduced the nasal blockage caused by R- $\alpha$ -methylhistamine, 600 $\mu$ g.
- 4) Co-administration with R- $\alpha$ -methylhistamine, 600 $\mu$ g, significantly reduced the nasal blockage caused by corynanthine, 2mg.
- 5) Pretreatment with topical thioperamide, 700 $\mu$ g, had no significant effect on the nasal blockage caused by histamine, 400 or 1000 $\mu$ g.
- 6) Pretreatment with a combination of oral cetirizine, 20mg, and topical thioperamide, 700 $\mu$ g, significantly reduced the nasal blockage caused by histamine, 1000 $\mu$ g, as compared to pretreatment with either antagonist alone.
- 7) The data indicate that histamine causes nasal blockage in normal individuals via the H<sub>3</sub>-mediated inhibition of basal sympathetic activity.

# CHAPTER 8

## GENERAL DISCUSSION AND FUTURE AIMS

The aim of this study was to investigate the roles of histamine receptors in the control of human nasal blockage. Nasal blockage occurs as a result of the engorgement of nasal sinusoids (Atkinson TP *et al.*, 1995) and, in this study, was measured objectively using acoustic rhinometry, as well as subjectively, using symptom scores. Acoustic rhinometry is a well-used and well-tolerated technique that uses acoustic reflection to map out the contours of the nasal cavity, thus giving a measure of the minimum cross-sectional area (Amin.) which is inversely proportional to nasal resistance (Hilberg O *et al.*, 1989). The reproducibility of this technique was demonstrated in Chapter 2. In addition to functional nasal measurements, nasal lavage and electrochemical high pressure liquid chromatography analysis were used in this study to assay the level of noradrenaline (NA) release from nasal sympathetic neurones. Nasal lavage is a quick and useful method of assessing the humoral and cellular activities in allergic rhinitis. It has the advantage of repeatability unlike the invasive and painful technique of nasal biopsy. However, there are limits to assaying the substances recovered in nasal lavages: incomplete lavage recovery, endogenous metabolism, venous clearance and the variable distribution of a substance throughout the nasal mucosa due to its chemical composition can hinder quantitative analyses.

Finally, it is important to remember this study investigated acute nasal blockage in humans and thus two factors should be considered. Firstly, all the studies investigated the nasal blockage caused by a single challenge of an inflammatory mediator. It may well be the case that the pathophysiological mechanisms involved in the clinical presentation of nasal blockage differ from the mechanisms described in this study. Secondly, due to ethical and safety considerations, there is obviously a limit to what experiments can be performed on the volunteers. This was particularly evident when deciding on the correct pharmacological tools to use.

Allergic rhinitis is an allergic disease of the upper airways that manifests itself in the symptoms of sneezing, pruritus, rhinorrhea and nasal congestion. These symptoms

are caused by the activation of neuronal, glandular and vascular mechanisms by the inflammatory mediators released following mast cell degranulation by the aeroallergen. One such inflammatory mediator is histamine (Naclerio RM *et al.*, 1983), which has been previously shown to mimic the symptoms of allergic rhinitis when sprayed onto the nasal mucosa (Miadonna A *et al.*, 1987; Mullins RJ *et al.*, 1989; Doyle WJ *et al.*, 1990; Rajakulasingam K *et al.*, 1993; Austin CE *et al.*, 1994c; Birchall MA *et al.*, 1996). In the present study, nasal challenge with histamine and pollen, in Chapters 3 and 5, respectively, has been shown to cause nasal blockage, pruritus, rhinorrhea and sneezing.

In Chapter 3, histamine-induced nasal blockage was shown to be reduced by selective H<sub>1</sub> antagonists, such as cetirizine and chlorpheniramine. However, for a given dose of histamine, in this case 400µg, administered as an aerosol into each nostril, it was demonstrated that pretreatment with cetirizine could not *abolish* the nasal blockage. Increasing the oral dose of cetirizine above 20mg did not further reduce the histamine-induced nasal blockage. Thus it was evident that histamine caused nasal blockage by at least two mechanisms: via H<sub>1</sub> receptors and via a non-H<sub>1</sub>-mediated mechanism.

In Chapter 4, the role of H<sub>2</sub> receptors in nasal blockage was investigated. Dimaprit, the selective H<sub>2</sub> agonist caused a small but significant nasal blockage which was abolished by pretreatment with ranitidine, the selective H<sub>2</sub> antagonist. In addition, ranitidine reduced, to a minor extent, the nasal blockage caused by histamine, although this appeared to be more consistent when the ranitidine was co-administered with the H<sub>1</sub> antagonist, cetirizine. Interestingly, pretreatment with maximal doses of H<sub>1</sub> and H<sub>2</sub> antagonists failed to abolish the nasal blockage caused by histamine, 400µg, in 3 out of 4 studies. The data in Chapter 4 suggested that although histamine causes nasal blockage via H<sub>1</sub> and H<sub>2</sub> receptors, there may be some interaction between these two receptor mechanisms. In addition, it appears that another, non-H<sub>1</sub>/H<sub>2</sub>, pathway is also responsible for a degree of the histamine-induced nasal blockage.

The basal role of nasal sympathetic neurones in the unchallenged nasal cavity was investigated in Chapter 6. Both NA and its metabolite, 3,4-dihydroxyphenylglycol (DHPG), were demonstrated in nasal lavages. In addition, antagonism of α<sub>1</sub>-

adrenoceptors caused nasal blockage, indicating an active role of NA in maintaining nasal patency. In Chapter 7, R- $\alpha$ -methylhistamine, the selective H<sub>3</sub> agonist, was shown to cause significant nasal blockage via the inhibition of basal NA release. By decreasing the release of NA, the H<sub>3</sub> agonist reduced basal vasoconstriction, causing indirect vasodilation and thus nasal blockage. This effect of R- $\alpha$ -methylhistamine was attributed to its activation of nasal H<sub>3</sub> receptors as (i) the nasal blockage was reduced following topical pretreatment with thioperamide, the H<sub>3</sub> antagonist; and (ii) the inactive isomer, S- $\alpha$ -methylhistamine, had no effect on nasal patency. It would be interesting to investigate the effect of R- $\alpha$ -methylhistamine on the levels of NA and DHPG in nasal lavages. The functional experiments suggest that R- $\alpha$ -methylhistamine would reduce NA and DHPG levels, an effect antagonized by pretreatment with thioperamide.

Interestingly, co-administration of R- $\alpha$ -methylhistamine with corynanthine did not cause greater nasal blockage than corynanthine alone. This observation suggests that the majority of basal sympathetic-induced vasoconstriction is mediated via the activation of vascular  $\alpha_1$ -adrenoceptors by NA. If other adrenoceptors or neuropeptide Y significantly contribute to maintaining nasal patency, then the inhibition of sympathetic activity by R- $\alpha$ -methylhistamine would have a greater effect than the inhibition of  $\alpha_1$ -adrenoceptors by corynanthine. Whether this  $\alpha_1$ -adrenoceptor dominance occurs in atopy is yet to be shown.

Whether histamine actually caused nasal blockage via H<sub>3</sub> receptors was harder to establish. Firstly, R- $\alpha$ -methylhistamine is 15 times more potent than histamine at H<sub>3</sub> receptors. Given that R- $\alpha$ -methylhistamine, 600 $\mu$ g, caused a 15% reduction in nasal patency, it is likely that the nasal blockage caused by histamine, 400 $\mu$ g, via H<sub>3</sub> receptors, may well be relatively small in effect. Secondly, thioperamide was unable, at the dose used (700 $\mu$ g, given as an aerosol into each nostril), to *abolish* the nasal blockage caused by R- $\alpha$ -methylhistamine (due to ethical and safety considerations, a higher dose of thioperamide was not studied). Thus, it is perhaps not surprising that the results for the thioperamide-induced reduction of nasal blockage caused by histamine, 400 $\mu$ g, were equivocal. However, by using a greater dose of histamine (1000 $\mu$ g), a thioperamide-sensitive component of the histamine-induced nasal blockage became significantly apparent, albeit only when the thioperamide was in combination with cetirizine, the H<sub>1</sub> antagonist. Overall, the data



in Chapter 7 suggested that, in addition to H<sub>1</sub> and H<sub>2</sub> receptor mechanisms, histamine also causes nasal blockage via activation of H<sub>3</sub> receptors.

In Chapter 5, the roles of H<sub>1</sub> and H<sub>2</sub> receptors in pollen-induced nasal responses were investigated. Cetirizine, 10mg, significantly reduced pollen-induced sneezing. In addition, pretreatment with cetirizine appeared to reduce the pollen-induced pruritus and rhinorrhea. However, neither cetirizine, nor a combination of cetirizine and ranitidine had any significant effect on pollen-induced nasal blockage (as measured by acoustic rhinometry and symptom scores). The data in Chapter 5 suggest that, although histamine (via activation of H<sub>1</sub> receptors) is clearly responsible for pollen-induced sneezing, pollen-induced nasal blockage is not mediated via H<sub>1</sub> or H<sub>2</sub> receptors. This observation is difficult to reconcile with the evidence in Chapter 3 and 4: that histamine can produce nasal blockage via the activation of these receptors. It has been shown on many occasions that histamine is released from nasal mast cells following allergen challenge (Naclerio RM *et al.*, 1983; Naclerio RM *et al.*, 1985; Miadonna A *et al.*, 1987; Walden SM *et al.*, 1988). The present study has shown that endogenous histamine is released in high enough levels to cause significant sneezing (and possibly pruritus and rhinorrhea) via H<sub>1</sub> receptors, which is in agreement with many other studies (Holmberg K *et al.*, 1989c; Naclerio RM, 1990; Bousquet J *et al.*, 1990; Vamey V, 1991; Falliers CJ *et al.*, 1991; Hilberg O *et al.*, 1995; Wood-Baker R *et al.*, 1996; Corren J *et al.*, 1999). In this way there seems to be little difference between histamine given exogenously and histamine released from mast cells by pollen: both should cause nasal blockage via H<sub>1</sub> (and possibly H<sub>2</sub>) receptors.

Why, then, do H<sub>1</sub> and H<sub>2</sub> antagonists fail to reduce pollen-induced nasal blockage? As discussed in Chapter 5.4, this is unlikely to be due to insufficient levels of these antagonists at nasal receptors. It is possible that, due to the release of other vasoactive substances following nasal challenge with pollen, the contribution of activated H<sub>1</sub> and H<sub>2</sub> receptors to pollen-induced nasal blockage is so small as to be difficult to observe without very large subject numbers. However, there is little evidence of other immediate-phase inflammatory mediators being responsible for pollen-induced nasal blockage. Although capable of causing nasal blockage when exogenously administered to the nasal cavity, bradykinin, leukotrienes and PGD<sub>2</sub> appear to play no significant role in pollen-induced nasal blockage (Brooks CD *et*

*al.*, 1984; Miadonna A *et al.*, 1987; Walden SM *et al.*, 1988; Doyle WJ *et al.*, 1990; Austin CE *et al.*, 1994b; Knapp HR *et al.*, 1994; Dear JW, 1996; Maynard ML *et al.*, 2001), although one study has shown a reduction of pollen-induced nasal blockage with an inhibitor of leukotriene synthesis (Knapp HR, 1990). Of course, each mediator may contribute a small (and difficult to elucidate) amount to the dilation of nasal blood vessels. Perhaps pollen-induced nasal blockage may only be inhibited by a combination of COX and 5-lipoxygenase inhibition, together with antagonists at bradykinin, leukotriene, H<sub>1</sub> and H<sub>2</sub> receptors. The role of neuropeptides in allergic rhinitis has been hampered by a lack of safe and selective pharmacological tools. As discussed in Chapter 1.7.1, neuropeptides appear not to contribute to the control of patency in the unchallenged nose. However, no studies investigating the effect of neuropeptide receptor antagonism in allergic rhinitis in humans have been reported, and so it is impossible to assess their role in pollen-induced nasal blockage.

The other possible explanation for the lack of sensitivity to H<sub>1</sub> and H<sub>2</sub> antagonists in allergic rhinitis is that histamine activation of non-H<sub>1</sub>/H<sub>2</sub> pathways is responsible for the pollen-induced nasal blockage. Histamine may stimulate the release of other vasoactive substances such as bradykinin or neuropeptides and it is possibly these substances that cause the vasodilation of nasal blood vessels. Histamine has been shown to increase the levels of kinins in nasal lavage (Svensson C *et al.*, 1989), but in another study, icatibant, the bradykinin B<sub>2</sub> receptor antagonist, failed to reduce histamine-induced nasal blockage (Turner PJ *et al.*, 2000a). A CGRP receptor antagonist has been shown to reduce histamine-induced nasal vasodilation in pigs (Malis DD *et al.*, 2001), however, in man, no increase in neuropeptide levels was recorded following nasal challenge with histamine (Mosimann BL *et al.*, 1993) and inhibition of neuropeptide breakdown has no effect on histamine-induced nasal blockage (Turner PJ, 1999). There are no reports of histamine increasing PGD<sub>2</sub> or leukotriene release. It is difficult, in fact, to conceive how histamine may cause the release of vasoactive mediators by a mechanism that is insensitive to histamine receptor antagonism.

Given that histamine causes nasal blockage via H<sub>3</sub> receptors, it is important to investigate the effect of thioperamide (or another H<sub>3</sub> antagonist) on pollen-induced nasal blockage. As discussed in Chapter 7.4, there may be some interaction between H<sub>1</sub> and H<sub>3</sub> receptors, resulting in a lack of significant effect of H<sub>3</sub>

antagonism when not given in combination with a H<sub>1</sub> antagonist. The data published by McLeod *et al.* (1999) demonstrated that feline nasal blockage caused by compound 48/80 (mast cell degranulator) was only inhibited by a *combination* of H<sub>1</sub> and H<sub>3</sub> antagonists. Perhaps co-administration with thioperamide may confer onto cetirizine effectiveness in reducing pollen-induced nasal blockage in man. If this is shown to be the case, it suggests that the H<sub>3</sub> receptor may contribute more to pollen-induced nasal blockage in atopic individuals than is observed in histamine-induced nasal blockage in normal individuals: the thioperamide-sensitive component of the nasal blockage caused by histamine, 400µg, was small in comparison to the cetirizine-sensitive component. Perhaps a greater role of H<sub>3</sub> receptors in atopic individuals may indicate H<sub>3</sub>-induced nasal blockage mediated via novel mechanisms, such as direct vasodilation caused by vascular H<sub>3</sub> receptors. Activation of vascular H<sub>3</sub> receptors has previously been shown to cause vasodilation in rabbit (Ea Kim L *et al.*, 1992) and cat blood vessels (Champion HC *et al.*, 1998), although neither study investigated sensitized (allergic) tissue. Alternatively, the thioperamide-sensitive component of the nasal blockage may be more significant in atopic individuals due to a disturbance in the normal function of nasal sympathetic neurones.

What might this disturbance be? For H<sub>3</sub> receptors to mediate a greater proportion of nasal blockage, it follows that the basal activity of sympathetic neurones must be increased in allergic rhinitis. As shown in Chapters 6 and 7, elimination of NA-induced nasal decongestion in normal individuals causes a 15% reduction in nasal patency. This reduction in nasal patency is not great enough to account for the extreme nasal blockage caused by pollen. However, if there is a chronic increase in sympathetic activity in atopic individuals, then perhaps H<sub>3</sub>-mediated reduction of NA release could have a greater effect on nasal patency. Malm *et al.* (1987) reported greater nasal blockage in atopic individuals than in normal individuals following nasal challenge with phentolamine, the α-adrenoceptor antagonist, suggesting increased basal sympathetic activity. Of course, this hypothesized increase in basal sympathetic activity would have the knock-on effect of increased nasal patency in atopic individuals. However, baseline (pre-challenge) measurements using rhinomanometry and acoustic rhinometry have not demonstrated increased nasal patency in atopic individuals compared with normal individuals (Devillier P *et al.*, 1988; Hilberg O *et al.*, 1995; Pirila T *et al.*, 1998; Takeno S *et al.*, 1998). Given that

nasal patency is not different in atopic individuals, there must also be a chronic increase in the tendency for nasal blood vessels to dilate in atopic individuals in order to balance the increase in sympathetic-mediated decongestion. As discussed in Chapter 6.4, nasal patency in the unchallenged individual is a balance between sympathetic mediators (vasoconstriction) and a passive vasodilatory pressure, which includes the hydrostatic pressure of the blood and possibly vasodilation mediated by endothelium-derived substances (such as NO and EDHF) or by substances released from parasympathetic neurones. It is possible that the basal vasoactive forces which control nasal patency are greater in atopic individuals, as shown in Figure 8.1.

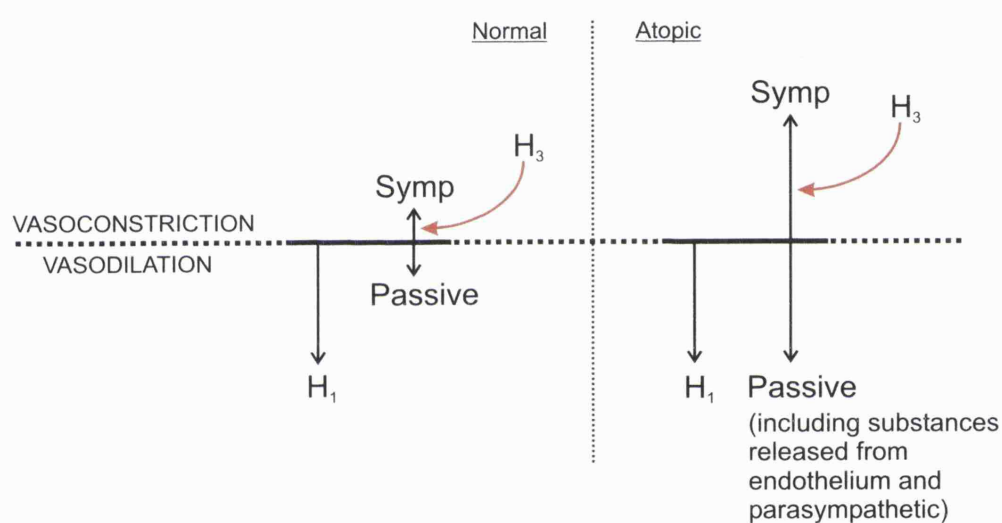


Figure 8.1: Hypothetical increase in basal vasoconstriction and vasodilation in allergy that may account for an increase in sensitivity to  $H_3$  receptor antagonism. Symp = vasoconstriction caused by substances released from nasal sympathetic neurones, Passive = passive vasodilation (caused by the hydrostatic pressure of blood and substances derived from vascular endothelium and parasympathetic neurones). Red arrows denote inhibition.

Hence, due to the limited role of the sympathetic nervous system in the control of nasal patency, the contribution of  $H_3$  receptors to nasal blockage in normal individuals is low. But with the hypothesized autonomic dysfunction in atopic individuals, activation of  $H_3$  receptors may have greater influence on nasal patency. In this way,  $H_3$  antagonism could prevent the inhibition (by mast cell-derived histamine) of the increased sympathetic activity, resulting in the release of *high* levels of sympathetic-derived vasoconstrictors and significantly reducing nasal blockage. It would be interesting to investigate the levels of NA and DHPG in nasal

lavages from atopic individuals, including lavages following allergen challenge with and without pretreatment with thioperamide.

The hypothesis illustrated in Figure 8.1 suggests that there may be three distinct mechanisms by which nasal blockage can occur: (i) direct vasodilation by inflammatory mediators, such as those released from mast cells and sensory neurones; (ii) inhibition of sympathetic activity, resulting in a decrease in vasoconstriction; and (iii) increased vasodilation caused by substances derived from vascular endothelium and parasympathetic neurones. In addition, it is possible that inhibition of only one of these mechanisms may be insufficient to reduce pollen-induced nasal blockage due to redundancy in the inflammatory process.

The theory depicted in Figure 8.1 suggests that there may be pathological changes in the function of the endothelium or parasympathetic nervous system in atopic individuals contributing to the increase in latent vasodilation. The roles of these two systems in the resting and the challenged nasal mucosa have not been fully identified. Inhibition of nitric oxide synthase or cyclooxygenase has no effect on resting nasal patency (McLean JA *et al.*, 1983; Dear JW *et al.*, 1996a). In addition, nitric oxide has been shown to mediate endothelium-dependent vasodilation following nasal challenge with bradykinin and house-dust mite, but not histamine nor pollen (Dear JW, 1996). There is little evidence to suggest a role of prostacyclin in mediating nasal endothelium-dependent vasodilation (McLean JA *et al.*, 1983; Brooks CD *et al.*, 1984; Walden SM *et al.*, 1988) and, unfortunately, there are no published studies that have investigated EDHF-mediated vasodilation in nasal mucosa. With respect to the parasympathetic nervous system, although atropine appears to have no effect on resting nasal patency or pollen-induced nasal blockage (Baroody FM *et al.*, 1994; Georgitis JW, 1998), the role of vasoactive intestinal peptide, a powerful vasodilator that is released following allergen challenge (Mosimann BL *et al.*, 1993), has yet to be understood. One particularly interesting observation is that *in vitro* stimulation of nasal cholinergic fibres can reduce the activity of sympathetic neurones (Wang HW *et al.*, 1988). It is possible that one or more of these mechanisms is augmented in atopic individuals. Again, redundancy amongst these mechanisms may hinder the understanding of the roles of the vascular endothelium and parasympathetic nervous system in the control of nasal patency.

Although most H<sub>1</sub> antagonists have been shown to have no effect on pollen-induced nasal blockage, there is a growing body of evidence demonstrating effective reduction of pollen-induced nasal blockage by desloratadine (Nayak AS *et al.*, 2001; Horak *et al.*, 2002; Schenkel E *et al.*, 2002; Wilson AM *et al.*, 2002). As discussed in Chapter 5.4, desloratadine is not significantly more potent than the other clinically-used H<sub>1</sub> antagonists. In fact, its selectivity for H<sub>1</sub> receptors is far lower than drugs such as cetirizine and fexofenadine (Gillard M *et al.*, 2003). To date, there are no reports of the affinity of desloratadine for H<sub>3</sub> receptors. It may be interesting to investigate the effect of desloratadine on R- $\alpha$ -methylhistamine-induced nasal blockage. In addition, there are no published reports of the inhibition by desloratadine of histamine-induced nasal blockage in normal individuals. If desloratadine, unlike cetirizine, the potent and selective H<sub>1</sub> antagonist, was shown to *abolish* histamine-induced nasal blockage, it could be postulated that the reduction of pollen-induced nasal blockage by desloratadine was due to its inhibition of undefined (non-H<sub>1</sub>/H<sub>2</sub>) histaminergic pathways. If this is shown not to be the case, the evidence suggests that the effectiveness of desloratadine in reducing pollen-induced nasal blockage is due to its inhibition of other, non-histaminergic, pathways. If so, these pathways offer novel therapeutic targets for treating allergic rhinitis. One such mechanism could be desloratadine's inhibition of both H<sub>1</sub> and muscarinic receptors (Gillard M *et al.*, 2003). Perhaps inhibition of muscarinic receptors reduces the latent vasodilatory mechanism described in Figure 8.1, thus conferring H<sub>1</sub> antagonist-sensitivity onto pollen-induced nasal blockage.

Much of the research into the pathophysiological mechanisms of nasal blockage has focused upon whether exogenous mediators *can* cause vasodilation. It is, perhaps, time that investigators attempt to discover whether or not specific substances *do* cause nasal blockage in allergic rhinitis. Clearly the development of selective receptor antagonists will aid future research. In addition, due to the possible redundancy of vasodilatory mechanisms, it may be beneficial to investigate the effects of combination treatments in allergic rhinitis.

In conclusion, this study has demonstrated that histamine causes nasal blockage via the activation of H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors. Further characterization of histamine receptors in the nasal mucosa may aid the understanding of these mechanisms. This study has also demonstrated the importance of basal sympathetic activity in

maintaining nasal patency in normal individuals. It is possible that the sympathetic nervous system may play a more important role in allergic rhinitis than has been previously considered. Both NA and NPY are potent vasoconstrictors (Wang SH *et al.*, 1993; Baraniuk JN, 1998) and NA has also been shown to inhibit histamine release from mast cells, albeit at high doses (Alm PE *et al.*, 1981). Although H<sub>1</sub> and H<sub>2</sub> antagonists fail to reduce pollen-induced nasal blockage, the H<sub>3</sub> receptor (its role in nasal blockage described here for the first time in humans *in vivo*), may offer a novel therapeutic target for the treatment of allergen-induced nasal blockage.

# CHAPTER 9

## REFERENCES

ABRAMS DA, ROBIN AL, POLLACK IP, DEFALLER JM, DESANTIS L (1987). The safety and efficacy of topical 1% ALO 2145 (p-aminoclonidine hydrochloride) in normal volunteers. *Arch Ophthalmol.* **105**(9):1205-7.

AGUILAR MI (2004). HPLC of peptides and proteins: basic theory and methodology. *Methods Mol Biol.* **251**:3-8.

AHMED T, SYRISTE T, MENDELSSOHN R, SORACE D, MANSOUR E, LANSING M, ABRAHAM WM, ROBINSON MJ (1994). Heparin prevents antigen-induced airway hyperresponsiveness: interference with IP<sub>3</sub>-mediated mast cell degranulation? *J Appl Physiol.* **76**(2):893-901.

AKDIS CA, BLASER K (2000). Mechanisms of allergen-specific immunotherapy. *Allergy.* **55**(6):522-30.

AKDIS CA, BLASER K (2003). Histamine in the immune regulation of allergic inflammation. *J Allergy Clin Immunol.* **112**(1):15-22.

ALBERT DH, MALO PE, TAPANG P, SHAUGHNESSY TK, MORGAN DW, WEGNER CD, CURTIN ML, SHEPPARD GS, XU L, DAVIDSEN SK, SUMMERS JB, CARTER GW (1998). The role of platelet-activating factor (PAF) and the efficacy of ABT-491, a highly potent and selective PAF antagonist, in experimental allergic rhinitis. *J Pharmacol Exp Ther.* **284**(1):83-8.

ALGUACIL LF, PEREZ-GARCIA C (2003). Histamine H<sub>3</sub> receptor: a potential drug target for the treatment of central nervous system disorders. *Curr Drug Target CNS Neurol Disord.* **2**(5):303-13.

ALM PE, BLOOM GD (1981). What - if any - is the role of adrenergic mechanisms in histamine release from mast cells? *Agents Actions.* **11**(1-2):60-6.

ALVING K, MATRAN R, LACROIX JS, LUNDBERG JM. (1990). Capsaicin and histamine antagonist-sensitive mechanisms in the immediate allergic reaction of pig airways. *Acta Physiol Scand.* **138**(1):49-60.

ALVING K, MATRAN R, LUNDBERG JM (1991). Effect of nedocromil sodium on allergen-, PAF-, histamine- and bradykinin-induced airways vasodilatation and pulmonary obstruction in the pig. *Br J Pharmacol.* **104**(2):452-8.

ANGGARD A (1977). Parasympathetic influence on the nasal mucosa. *Acta Otolaryngol.* **83**(1-2):22-4.

ANICHTCHIK OV, PEITSARO N, RINNE JO, KALIMO H, PANULA P (2001). Distribution and modulation of histamine H<sub>3</sub> receptors in basal ganglia and frontal cortex of healthy controls and patients with Parkinson's disease. *Neurobiol Dis.* **8**(4):707-16.

ARCHER SL, COWAN NJ (1991). Measurement of endothelial cytosolic calcium concentration and nitric oxide production reveals discrete mechanisms of endothelium-dependent pulmonary vasodilatation. *Circ Res.* **68**(6):1569-81.



- ARIAS-MONTANO JA, FLORAN B, GARCIA M, ACEVES J, YOUNG JM (2001). Histamine H<sub>3</sub> receptor-mediated inhibition of depolarization-induced, dopamine D<sub>1</sub> receptor-dependent release of [<sup>3</sup>H]-gamma-aminobutyric acid from rat striatal slices. *Br J Pharmacol*. **133**(1):165-71.
- ARIHOOD SA, JACKSON RT (1976). Norepinephrine-epinephrine and serotonin content of nasal mucosa in relation to hemorrhage, reserpine and excitement. *Life Sci*. **18**(2):211-4.
- ARRANG JM, GARBARG M, LANCELOT JC, LECOMTE JM, POLLARD H, ROBBA M, SCHUNACK W, SCHWARTZ JC (1987). Highly potent and selective ligands for histamine H<sub>3</sub>-receptors. *Nature*. **327**(6118):117-23.
- ARRANG JM, GARBARG M, SCHWARTZ JC (1983). Auto-inhibition of brain histamine release mediated by a novel class (H<sub>3</sub>) of histamine receptor. *Nature*. **302**(5911):832-7.
- ARRANG JM, GARBARG M, SCHWARTZ JC, LIPP R, STARK H, SCHUNACK W, LECOMTE JM (1991). The histamine H<sub>3</sub>-receptor: pharmacology, roles and clinical implications studied with agonists. *Agents Actions Suppl*. **33**:55-67.
- ASH AS, SCHILD HO (1966). Receptors mediating some actions of histamine. *Br J Pharmacol*. **27**:427-39.
- ATKINSON TP, KALINER MA (1995). Vascular mechanisms in rhinitis. *Asthma and Rhinitis* (Busse W, Holgate S eds). 777-88.
- AUSTIN CE, DEAR JW, NEIGHBOUR H, LUND V, FOREMAN JC (1996). The contribution of histamine to the action of bradykinin in the human nasal airway. *Immunopharmacology*. **34**(2-3):181-9.
- AUSTIN CE, FOREMAN JC (1993). The effect of platelet-activating factor on the responsiveness of the human nasal airway. *Br J Pharmacol*. **110**(1):113-8.
- AUSTIN CE, FOREMAN JC (1994a). A study of the action of bradykinin and bradykinin analogues in the human nasal airway. *J Physiol*. **478** (2):351-6.
- AUSTIN CE, FOREMAN JC, SCADDING GK (1994b). Reduction by Hoe 140, the B<sub>2</sub> kinin receptor antagonist, of antigen-induced nasal blockage. *Br J Pharmacol*. **111**(4):969-71.
- AUSTIN CE, FOREMAN JC. (1994c). Acoustic rhinometry compared with posterior rhinomanometry in the measurement of histamine- and bradykinin-induced changes in nasal airway patency. *Br J Clin Pharmacol*. **37**(1):33-7.
- BABHAIR SA, TARIQ M, ABDULLAH ME (1990). Comparison of intravenous and nasal bioavailability of clonidine in rodents. *Res Commun Chem Pathol Pharmacol*. **67**(2):241-8.
- BACHERT C, WAGENMANN M, HOLTAPPELS G (1998). Cytokines and adhesion molecules in allergic rhinitis. *Am J Rhinol*. **12**(1):3-8.
- BARANIUK JN (1992). Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa. *J Allergy Clin Immunol*. **90**(6 Pt 2):1045-50.
- BARANIUK JN (1997). Pathogenesis of allergic rhinitis. *J Allergy Clin Immunol*. **99**(2):S763-72.
- BARANIUK JN (1998). Neuropeptides. *Am J Rhinol*. **12**(1):9-16.

- BARANIUK JN, SILVER PB, KALINER MA, BARNES PJ (1992). Neuropeptide Y is a vasoconstrictor in human nasal mucosa. *J Appl Physiol.* **73**(5):1867-72.
- BARNES PJ (1990). Neuropeptides as modulators of airway function. *Agents Actions Suppl.* **31**:175-96.
- BARNES PJ (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond).* **94**(6):557-72.
- BAROODY FM, FORD S, LICHTENSTEIN LM, KAGEY-SOBOTKA A, NACLERIO RM. (1994). Physiologic responses and histamine release after nasal antigen challenge. Effect of atropine. *Am J Respir Crit Care Med.* **149**(6):1457-65.
- BASBAUM AI, LEVINE JD (1991). The contribution of the nervous system to inflammation and inflammatory disease. *Can J Physiol Pharmacol.* **69**(5):647-51.
- BAUMGARTEN CR, NICHOLS RC, NACLERIO RM, LICHTENSTEIN LM, NORMAN PS, PROUD D (1986a). Plasma kallikrein during experimentally induced allergic rhinitis: role in kinin formation and contribution to TAME-esterase activity in nasal secretions. *J Immunol.* **137**(3):977-82.
- BAUMGARTEN CR, NICHOLS RC, NACLERIO RM, PROUD D (1986b). Concentrations of glandular kallikrein in human nasal secretions increase during experimentally induced allergic rhinitis. *J Immunol.* **137**(4):1323-8.
- BAUMGARTEN CR, O'CONNOR A, DOKIC D, SCHULTZ KD, KUNKEL G (1997). Substance P is generated in vivo following nasal challenge of allergic individuals with bradykinin. *Clin Exp Allergy.* **27**(11):1322-7.
- BAUMGARTEN CR, WITZEL A, KLEINE-TEBBE J, KUNKEL G (1996). Substance P enhances antigen-evoked mediator release from human nasal mucosa. *Peptides.* **17**(1):25-30.
- BENSON M, STRANNEGARD IL, STRANNEGARD O, WENNERGREN G (2000). Topical steroid treatment of allergic rhinitis decreases nasal fluid T<sub>H2</sub> cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN-gamma, IL-1beta, TNF-alpha, or neutrophils. *J Allergy Clin Immunol.* **106**(2):307-12.
- BERGER WE (2003). Overview of allergic rhinitis. *Ann Allergy Asthma Immunol.* **90**(6 Suppl 3):7-12.
- BERNHEIM J, ARENDT C, DE VOS C (1991). Cetirizine: more than an antihistamine? *Agents Actions Suppl.* **34**:269-93.
- BERTACCINI G, CORUZZI G, POLI E, ADAMI M (1986). Pharmacology of the novel H<sub>2</sub> antagonist famotidine: in vitro studies. *Agents Actions.* **19**(3-4): 180-7.
- BEYAK M, VANNER S (1995). Histamine H<sub>1</sub> and H<sub>3</sub> vasodilator mechanisms in the guinea pig ileum. *Gastroenterology.* **108**(3):712-8.
- BICKFORD L, SHAKIB S, TAVERNER D. (1999). The nasal airways response in normal subjects to oxymetazoline spray: randomized double-blind placebo-controlled trial. *Br J Clin Pharmacol.* **48**(1):53-6.
- BIRCHALL MA, HENDERSON JC, PRIDE NB, FULLER RW (1996). A comparison of the effects of an alpha-agonist, an anti-muscarinic agent and placebo on intranasal histamine challenge in allergic rhinitis. *Clin Otolaryngol.* **21**(3):212-7.

- BIRCHALL MA, SCHROTER RC, PRIDE NB (1993). Changes in nasal mucosal blood flux and air-flow resistance on unilateral histamine challenge. *Clin Otolaryngol.* **18**(2):139-44.
- BLACK JW, DUNCAN WA, DURANT CJ, GANELLIN CR, PARSONS EM (1972). Definition and antagonism of histamine H<sub>2</sub>-receptors. *Nature.* **236**(5347):385-90.
- BLANDIZZU C, COLUCCI R, TOGNETTI M, DE PAOLIS B, DEL TACCA M (2001). H<sub>3</sub> receptor-mediated inhibition of intestinal acetylcholine release: pharmacological characterization of signal transduction pathways. *Naunyn Schmiedebergs Arch Pharmacol.* **363**(2):193-202.
- BLANDIZZU C, TOGNETTI M, COLUCCI R, DEL TACCA M (2000). Histamine H<sub>3</sub> receptors mediate inhibition of noradrenaline release from intestinal sympathetic nerves. *Br J Pharmacol.* **129**(7):1387-96.
- BOCKMAN CS, ZENG W (2002). Histamine receptor type coupled to nitric oxide-induced relaxation of guinea-pig nasal mucosa. *Auton Autacoid Pharmacol.* **22**(5-6):269-76.
- BOEHM S, HUCK S (1997). Receptors controlling transmitter release from sympathetic neurons in vitro. *Prog Neurobiol.* **51**(3):225-42.
- BOOTH RG, MONIRI NH, BAKKER RA, CHOKSI NY, NIX WB, TIMMERMAN H, LEURS R (2002). A novel phenylaminotetralin radioligand reveals a subpopulation of histamine H<sub>1</sub> receptors. *J Pharmacol Exp Ther.* **302**(1):328-36.
- BOUSQUET J, CHANEZ P, MICHEL FB (1990). Pathophysiology and treatment of seasonal allergic rhinitis. *Respir Med.* **84** Suppl A:11-7.
- BOUSQUET J, VIGNOLA AM, CAMPBELL AM, MICHEL FB (1996). Pathophysiology of allergic rhinitis. *Int Arch Allergy Immunol.* **110**(3):207-18.
- BOVET D (1950). Introduction to antihistamine agents and Antergan derivatives. *Ann NY Acad Sci.* **50**:1089-1126.
- BRANCO D, CARAMONA M, ARAUJO D, OSSWALD W (1992). The role of monoamine oxidase in the metabolism of exogenous noradrenaline by the human saphenous vein. *Fundam Clin Pharmacol.* **6**(3):145-52.
- BRAUNSTAHL GJ, OVERBEEK SE, KLEINJAN A, PRINS JB, HOOGSTEDEN HC, FOKKENS WJ (2001). Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol.* **107**(3):469-76.
- BRAUNSTEIN G, FAJAC I, LACRONIQUE J, FROSSARD N (1991). Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. *Am Rev Respir Dis.* **144**(3 Pt 1):630-5.
- BRODY MJ (1980). Histamine and vascular smooth muscle. *Vascular neuroeffector mechanisms.* Eds Bevan. 82-6.
- BROOKS CD, NELSON AL, METZLER C (1984). Effect of flurbiprofen, a cyclooxygenase inhibiting drug, on induced allergic rhinitis. *J Allergy Clin Immunol.* **73**(5 Pt 1):584-9.
- BUCKLAND KF, WILLIAMS TJ, CONROY DM (2003). Histamine induces cytoskeletal changes in human eosinophils via the H<sub>4</sub> receptor. *Br J Pharmacol.* **140**(6):1117-27.
- BUSSE R, EDWARDS G, FELETOU M, FLEMING I, VANHOUTTE PM, WESTON AH (2002). EDHF: bringing the concepts together. *Trends Pharmacol Sci.* **23**(8):374-80.

- BUSSE R, FORSTERMANN U, MATSUDA H, POHL U (1984). The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia. *Pflügers Arch.* **401**(1):77-83.
- BUSSE WW (1996). The role of leukotrienes in asthma and allergic rhinitis. *Clin Exp Allergy.* **26**(8):868-79.
- CAINE M, PERLBERG S, SHAPIRO A (1981). Phenoxybenzamine for benign prostatic obstruction. Review of 200 cases. *Urology.* **17**(6):542-6.
- CALLAWAY JK, KING RG, BOURA AL (1990). Methoxyphenamine inhibits histamine-induced bronchoconstriction in anaesthetized guinea-pigs and histamine-induced contractions of guinea-pig ileum in vitro. *Arch Int Pharmacodyn Ther.* **308**:86-94.
- CAMPOLI-RICHARDS DM, BUCKLEY MM, FITTON A (1990). Cetirizine. A review of its pharmacological properties and clinical potential in allergic rhinitis, pollen-induced asthma, and chronic urticaria. *Drugs.* **40**(5):762-81.
- CANONICA GW, PASSALACQUA G (2003). Noninjection routes for immunotherapy. *J Allergy Clin Immunol.* **111**(3):437-48.
- CASALE TB (2001). Anti-immunoglobulin E (omalizumab) therapy in seasonal allergic rhinitis. *Am J Respir Crit Care Med.* **164**(8 Pt 2):S18-21.
- CASTERLINE CL, EVANS R (1977). Further studies on the mechanism of human histamine-induced asthma: the effect of an aerosolized H<sub>1</sub> receptor antagonist (diphenhydramine). *J Allergy Clin Immunol.* **59**(6):420-4.
- CELUCH SM (1995). Possible participation of histamine H<sub>3</sub> receptors in the modulation of noradrenaline release from rat spinal cord slices. *Eur J Pharmacol.* **287**(2):127-33.
- CHAMPION HC, KADOWITZ PJ (1998). R(-)-alpha-methyl-histamine has nitric oxide-mediated vasodilator activity in the mesenteric vascular bed of the cat. *Eur J Pharmacol.* **343**(2-3):209-16.
- CHANG JY, HARDEBO JE, OWMAN C (1988). Differential vasomotor action of noradrenaline, serotonin, and histamine in isolated basilar artery from rat and guinea-pig. *Acta Physiol Scand.* **132**(1):91-102.
- CHAPLEO CB, BUTLER RC, ENGLAND DC, MYERS PL, ROACH AG, SMITH CF, STILLINGS MR, TULLOCH IF (1989). Heteroaromatic analogues of the alpha<sub>2</sub>-adrenoreceptor partial agonist clonidine. *J Med Chem.* **32**(7):1627-30.
- CHATELAIN C, POCHON N, LACROIX JS (1995). Functional effects of phosphoramidon and captopril on exogenous neuropeptides in human nasal mucosa. *Eur Arch Otorhinolaryngol.* **252**(2):83-5.
- CHEN GF, SUZUKI H (1990). Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. *J Physiol.* **421**:521-34.
- CHEN Y, GETCHELL TV, SPARKS DL, GETCHELL ML (1993). Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: age-related trends. *J Comp Neurol.* **334**(1):104-16.
- CHERVINSKY P, CASALE T, TOWNLEY R, TRIPATHY I, HEDGECOCK S, FOWLER-TAYLOR A, SHEN H, FOX H (2003). Omalizumab, an anti-IgE antibody, in the treatment of adults and adolescents with perennial allergic rhinitis. *Ann Allergy Asthma Immunol.* **91**(2):160-7.

- CHEUNG D, VAN DER VEEN H, DEN HARTIGH J, DIJKMAN JH, STERK PJ (1994). Effects of inhaled substance P on airway responsiveness to methacholine in asthmatic subjects in vivo. *J Appl Physiol.* **77**(3):1325-32.
- CHIBA Y, MISAWA M (1994). Antigen-induced airway hyperresponsiveness is associated with airway tissue NEP hypoactivity in rats. *Life Sci.* **55**(24):1919-28.
- CHRISTIANSEN SC, EDDLESTON J, WOESSNER KM, CHAMBERS SS, YE R, PAN ZK, ZURAW BL (2002). Up-regulation of functional kinin B<sub>1</sub> receptors in allergic airway inflammation. *J Immunol.* **169**(4):2054-60.
- CHURCHILL L, PONGRACIC JA, REYNOLDS CJ, NACLERIO RM, PROUD D (1991). Pharmacology of nasal provocation with bradykinin: studies of tachyphylaxis, cyclooxygenase inhibition, alpha-adrenergic stimulation, and receptor subtype. *Int Arch Allergy Appl Immunol.* **95**(4):322-31.
- COGE F, GUENIN SP, AUDINOT V, RENOUEAU-TRY A, BEAUVERGER P, MACIA C, OUVRY C, NAGEL N, RIQUE H, BOUTIN JA, GALIZZI JP (2001). Genomic organization and characterization of splice variants of the human histamine H<sub>3</sub> receptor. *Biochem J.* **355**(Pt 2):279-88.
- COLE P, HAIGHT JS, COOPER PW, KASSEL EE (1983). A computed tomographic study of nasal mucosa: effects of vasoactive substances. *J Otolaryngol.* **12**(1):58-60.
- CORRADO OJ, OLLIER S, PHILLIPS MJ, THOMAS JM, DAVIES RJ (1987). Histamine and allergen induced changes in nasal airways resistance measured by anterior rhinomanometry: reproducibility of the technique and the effect of topically administered antihistaminic and anti-allergic drugs. *Br J Clin Pharmacol.* **24**(3):283-92.
- CORREN J, RACHELEFSKY G, SPECTOR S, SCHANKER H, SIEGEL S, HOLTON D, KARCHER K, TRAVERS S (1999). Onset and duration of action of levocabastine nasal spray in atopic patients under nasal challenge conditions. *J Allergy Clin Immunol.* **103**(4):574-80.
- COSTELLO RW, EVANS CM, YOST BL, BELMONTE KE, GLEICH GJ, JACOBY DB, FRYER AD (1999). Antigen-induced hyperreactivity to histamine: role of the vagus nerves and eosinophils. *Am J Physiol.* **276**(5 Pt 1):L709-14.
- COVINO BG (1987). Toxicity and systemic effects of local anaesthetic agents. *Handb Exp Pharm.* **81**:187-212.
- COYLE AJ, ACKERMAN SJ, IRVIN CG (1993). Cationic proteins induce airway hyperresponsiveness dependent on charge interactions. *Am Rev Respir Dis.* **147**(4):896-900.
- CRIMI N, ARMATO E, LAMICELA M, MAGRI S, PISTORIO MP, MASTRUZZO C, VANCHERI C (1995). Inhibitory effect of heparin on eosinophils recruitment after nasal allergen challenge. *Eur Resp J.* **8**:10S.
- DACHMAN WD, BEDARIDA G, BLASCHKE TF, HOFFMAN BB (1994). Histamine-induced venodilation in human beings involves both H<sub>1</sub> and H<sub>2</sub> receptor subtypes. *J Allergy Clin Immunol.* **93**(3):606-14.
- DALE HH (1929). Some chemical factors in the control of the circulation. *Lancet.* **i**: 1179-1183, 1233-1237, 1285-1290.

- DALE HH, LAIDLAW PP (1910). The physiological action of beta-imidazolylethylamine. *J Physiol.* **41**:318-44.
- DANKO G, HEY JA, EGAN RW, KREUTNER W, CHAPMAN RW (1994). Histamine H<sub>3</sub> receptors inhibit sympathetic modulation of airway microvascular leakage in allergic guinea pigs. *Eur J Pharmacol.* **254**(3):283-6.
- DAVIES MG, GREAVES MW (1980). Sensory responses of human skin to synthetic histamine analogues and histamine. *Br J Clin Pharmacol.* **9**(5):461-5.
- DAWES JD, PRICHARD MM (1953). Studies of the vascular arrangements of the nose. *J Anat.* **87**(3):311-22.
- DE LA LANDE IS, PARKER DA, PROCTOR CH, MARINO V, MACKAY-SIM A (1987). Cocaine inhibits extraneuronal O-methylation of exogenous norepinephrine in nasal and oral tissues of the rabbit. *Life Sci.* **41**(22):2463-8.
- DE SOUSA MUCIDA D, DE CASTRO KELLER A, FERNVIK EC, RUSSO M (2003). Unconventional strategies for the suppression of allergic asthma. *Curr Drug Targets Inflamm Allergy.* **2**(2):187-95.
- DE VRIES A, VAN RIJNSOEVER C, ENGELS F, HENRICKS P, NIJKAMP FP (2001). The role of sensory nerve endings in nerve growth factor-induced airway hyperresponsiveness to histamine in guinea-pigs. *Br J Pharmacol.* **134**: 771-776.
- DEAR JW (1996). The bradykinin receptor in the human nasal airway: its characteristics and role in allergic rhinitis. *UCL PhD Thesis.*
- DEAR JW, GHALI S, FOREMAN JC (1996a). Attenuation of human nasal airway responses to bradykinin and histamine by inhibitors of nitric oxide synthase. *Br J Pharmacol.* **118**(5):1177-82.
- DEAR JW, WIRTH K, SCADDING GK, FOREMAN JC (1996b). Characterization of the bradykinin receptor in the human nasal airway using the binding of [<sup>125</sup>I]-Hoe 140. *Br J Pharmacol.* **119**(5):1054-62.
- DEINHOFFER K, SEVCIK H, BALIC N, HARWANEGG C, HILLER R, RUMPOLD H, MUELLER MW, SPITZAUER S (2004). Microarrayed allergens for IgE profiling. *Methods.* **32**(3):249-54.
- DESAI HG, BORKAR AV, JEEJEEBHOY KN (1967). Dose-weight relationship of histamine for maximal stimulation of gastric acid secretion. *Gastroenterology.* **53**(5): 712-8.
- DEVILLIER P, DESSANGES JF, RAKOTOSIHANAKA F, GHAEM A, BOUSHEY HA, LOCKHART A, MARSAC J (1988). Nasal response to substance P and methacholine in subjects with and without allergic rhinitis. *Eur Respir J.* **1**(4):356-61.
- DICPINIGAITIS PV, DOBKIN JB (1996). Effect of angiotensin-converting enzyme inhibition on bronchial responsiveness. *J Clin Pharmacol.* **36**(4):361-4.
- DOUGLASS JA, DHAMI D, GURR CE, BULPITT M, SHUTE JK, HOWARTH PH, LINDLEY IJ, CHURCH MK, HOLGATE ST (1994). Influence of interleukin-8 challenge in the nasal mucosa in atopic and nonatopic subjects. *Am J Respir Crit Care Med.* **150**(4):1108-13.
- DOXEY JC, LANE AC, ROACH AG, VIRDEE NK (1984). Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781094), yohimbine, rauwolscine and corynanthine. *Naunyn Schmiedebergs Arch Pharmacol.* **325**(2):136-44.

- DOYLE WJ, BOEHM S, SKONER DP (1990). Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin, and prostaglandin in adult volunteers with and without nasal allergy. *J Allergy Clin Immunol.* **86**(6 Pt 1):924-35.
- DOYLE WJ, SKONER DP, SEROKY JT, FIREMAN P (1995). Reproducibility of the effects of intranasal ragweed challenges in allergic subjects. *Ann Allergy Asthma Immunol.* **74**(2):171-6.
- DRAGSTEDT CA, GEBAUER-FUELNEGG E (1932). Studies in anaphylaxis 1. The appearance of a physiologically active substance during anaphylactic shock. *Am J Physiol.* **102**:512-19.
- DUBUSKE L (1995). Dose-ranging comparative evaluation of cetirizine in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol.* **74**(4):345-54.
- DURHAM SR, KAY AB (1985). Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy.* **15**(5): 411-8.
- DURHAM SR, TILL SJ (1998). Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol.* **102**(2):157-64.
- EA KIM L, JAVELLAUD J, OUDART N (1992). Endothelium-dependent relaxation of rabbit middle cerebral artery to a histamine H<sub>3</sub>-agonist is reduced by inhibitors of nitric oxide and prostacyclin synthesis. *Br J Pharmacol.* **105**(1):103-6.
- ECCLES R (2000). Nasal airflow in health and disease. *Acta Otolaryngol.* **120**(5):580-95.
- ECCLES R, JAWAD MS, MORRIS S (1990). The effects of oral administration of (-)-menthol on nasal resistance to airflow and nasal sensation of airflow in subjects suffering from nasal congestion associated with the common cold. *J Pharm Pharmacol.* **42**(9):652-4.
- EDVINSSON L (2001). Sensory nerves in man and their role in primary headaches. *Cephalalgia.* **21**(7):761-4.
- EDWARDS AD, MANICKASINGHAM SP, SPORRI R, DIEBOLD SS, SCHULZ O, SHER A, KAISHO T, AKIRA S, REIS E, SOUSA C (2002). Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J Immunol.* **169**(7):3652-60.
- EISENBARTH SC, PIGGOTT DA, BOTTOMLY K (2003). The master regulators of allergic inflammation: dendritic cells in T<sub>H2</sub> sensitization. *Curr Opin Immunol.* **15**(6):620-6.
- EISENHOFER G, ESLER MD, MEREDITH IT, DART A, CANNON RO, QUYYUMI AA, LAMBERT G, CHIN J, JENNINGS GL, GOLDSTEIN DS (1992). Sympathetic nervous function in human heart as assessed by cardiac spillovers of dihydroxyphenylglycol and norepinephrine. *Circulation.* **85**(5):1775-85.
- ELLENBOGEN KA, SMITH ML, BEIGHTOL LA, ECKBERG DL (1992). Influence of lidocaine on human muscle sympathetic nerve activity during programmed electrical stimulation and ventricular tachycardia. *Am Heart J.* **124**(4):891-7.
- EMERY BE, WHITE MV, IGARASHI Y, MULLOL J, BERKEBILE C, PEDEN D, LOTZE MT, KALINER MA (1992). The effect of IL-4 on human nasal mucosal responses. *J Allergy Clin Immunol.* **90**(5):772-81.
- ENERBACK L, PIPKORN U, GRANERUS G (1986). Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int Arch Allergy Appl Immunol.* **80**(1):44-51.

- ENNIS M (1982). Histamine release from human pulmonary mast cells. *Agents Actions*. **12**(1-2):60-3.
- ERJEFALT JS, GREIFF L, ANDERSSON M, MATSSON E, PETERSEN H, LINDEN M, ANSARI T, JEFFERY PK, PERSSON CG (1999). Allergen-induced eosinophil cytotoxicity is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med*. **160**(1):304-12.
- ESLER M (1993). Clinical application of noradrenaline spillover methodology: delineation of regional human sympathetic nervous responses. *Pharmacol Toxicol*. **73**(5):243-53.
- EVANS CM, BELMONTE KE, COSTELLO RW, JACOBY DB, GLEICH GJ, FRYER AD (2000). Substance P-induced airway hyperreactivity is mediated by neuronal M<sub>2</sub> receptor dysfunction. *Am J Physiol Lung Cell Mol Physiol*. **279**(3):L477-86.
- EVANS CM, FRYER AD, JACOBY DB, GLEICH GJ, COSTELLO RW (1997). Pretreatment with antibody to eosinophil major basic protein prevents hyperresponsiveness by protecting neuronal M<sub>2</sub> muscarinic receptors in antigen-challenged guinea pigs. *J Clin Invest*. **100**(9):2254-62.
- FAJAC I, BRAUNSTEIN G, ICKOVIC MR, LACRONIQUE J, FROSSARD N (1995). Selective recruitment of eosinophils by substance P after repeated allergen exposure in allergic rhinitis. *Allergy*. **50**(12):970-5.
- FALCONER AD, LAKE D, MACDONALD IA (1982). The measurement of plasma noradrenaline by high-performance liquid chromatography with electrochemical detection: an assessment of sample stability and assay reproducibility. *J Neurosci Methods*. **6**(3):261-7.
- FALLIERS CJ, BRANDON ML, BUCHMAN E, CONNELL JT, DOCKHORN R, LEESE PT, MILLER J, WASSERMAN SI, ZETERBERG JM, ALTMAN R (1991). Double-blind comparison of cetirizine and placebo in the treatment of seasonal rhinitis. *Ann Allergy*. **66**(3):257-62.
- FANG SY, DRUCE HM, BARANIUK JN (1998). Anticholinergic properties of brompheniramine, chlorpheniramine, and atropine in human nasal mucosa in vitro. *Am J Rhinol*. **12**(2):131-3.
- FELDBERG W (1953). Recent experiments about histamine and allergic reactions. *Proc R Soc Med*. **46**(4):256-60.
- FERGUSON RK, ROTHENBERG RJ, NIES AS (1976). Patient acceptance of guanethidine as therapy for mild to moderate hypertension. A comparison with reserpine. *Circulation*. **54**(1):32-7.
- FISHER EW, LUND VJ, SCADDING GK (1994). Acoustic rhinometry in rhinological practice: discussion paper. *J R Soc Med*. **87**(7):411-3.
- FLEIG HA, PATIL PN, KRIEGLSTEIN GK (1987). The neuronal and extraneuronal uptake and metabolism of <sup>3</sup>H-(-)-noradrenaline in the rabbit iris. *Int Ophthalmol*. **10**(1):15-22.
- FLORES PEREZ J, JUAREZ OLGUIN H, FLORES PEREZ C, PEREZ GUILLE G, GUILLE PEREZ A, CAMACHO VIEYRA A, TOLEDO LOPEZ A, CARRASCO PORTUGAL M, LARES ASSEFF I (2003). Effects of gender and phase of the menstrual cycle on the kinetics of ranitidine in healthy volunteers. *Chronobiol Int*. **20**(3):485-94.



- FOSTER PS, GOLDIE RG, PATERSON JW (1983). Effect of steroids on beta-adrenoceptor-mediated relaxation of pig bronchus. *Br J Pharmacol*. **78**(2):441-5.
- FUJIWARA M, USUI H, KURAHASHI K, JINO H, SHIRAHASE H, MEKATA F (1992). Endothelium-dependent contraction produced by acetylcholine and relaxation produced by histamine in monkey basilar arteries. *J Cardiovasc Pharmacol*. **20 Suppl 12**:S114-6.
- GALE AE, SOLOMON E, TAO BS (1980). Intranasal topical flunisolide therapy in children with seasonal allergic rhinitis. *Clin Allergy*. **10**(5):527-33.
- GANGUR V, BIRMINGHAM NP, THANESVORAKUL S, JOSEPH S (2003). CCR3 and CXCR3 as drug targets for allergy: principles and potential. *Curr Drug Targets Inflamm Allergy*. **2**(1):53-62.
- GARBARG M, ARRANG JM, ROULEAU A, LIGNEAU X, TUONG MD, SCHWARTZ JC, GANELLIN CR (1992). S-[2-(4-imidazolyl)ethyl]isothiurea, a highly specific and potent histamine H<sub>3</sub> receptor agonist. *J Pharmacol Exp Ther*. **263**(1):304-10.
- GEHA RS (2003). Allergy and hypersensitivity. Nature versus nurture in allergy and hypersensitivity. *Curr Opin Immunol*. **15**(6):603-8.
- GEHA RS, JABARA HH, BRODEUR SR (2003). The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol*. **3**(9):721-732.
- GEORGITIS JW (1998). Nasal atropine sulfate: efficacy and safety of 0.050% and 0.075% solutions for severe rhinorrhea. *Arch Otolaryngol Head Neck Surg*. **124**(8):916-20.
- GERARD NP, GERARD C. (2002). Complement in allergy and asthma. *Curr Opin Immunol*. **14**(6):705-8.
- GIARDINA GA, GAGLIARDI S, MARTINELLI M (2003). Antagonists at the neurokinin receptors--recent patent literature. *IDrugs*. **6**(8):758-72.
- GILLARD M, CHRISTOPHE B, WELS B, PECK M, MASSINGHAM R, CHATELAIN P (2003). H<sub>1</sub> antagonists: receptor affinity versus selectivity. *Inflamm Res*. **52 Suppl 1**:S49-50.
- GOMEZ E, CORRADO OJ, DAVIES RJ (1987). Histochemical and functional characteristics of the human nasal mast cell. *Int Arch Allergy Appl Immunol*. **83**(1):52-6.
- GOTOH M (1999). Role of angiotensin-converting enzyme on bradykinin nasal provocation: comparison of normal volunteers and allergic subjects. *Nippon Jibiinkoka Gakkai Kaiho*. **102**(2):218-25.
- GREENBERGER PA (2002). Immunotherapy update: mechanisms of action. *Allergy Asthma Proc*. **23**(6):373-6.
- GREVERS G, KASTENBAUER E (1996). Functional morphology of nasal blood vessels in humans. *Acta Otolaryngol*. **116**(2):312-5.
- GRIMM M, WEIDMANN P, KEUSCH G, MEIER A, GLUCK Z (1980). Norepinephrine clearance and pressor effect in normal and hypertensive man. *Klin Wochenschr*. **58**(21):1175-81.
- GROSS PM (1981). Histamine H<sub>1</sub>- and H<sub>2</sub>-receptors are differentially and spatially distributed in cerebral vessels. *J Cereb Blood Flow Metab*. **1**(4):441-6.

- GROSS PM, HARPER AM, TEASDALE GM (1981a). Cerebral circulation and histamine: 1. Participation of vascular H<sub>1</sub>- and H<sub>2</sub>-receptors in vasodilatory responses to carotid arterial infusion. *J Cereb Blood Flow Metab.* **1**(1):97-108.
- GROSS PM, HARPER AM, TEASDALE GM (1981b). Cerebral circulation and histamine: 2. Responses of pial veins and arterioles to receptor agonists. *J Cereb Blood Flow Metab.* **1**(2):219-25.
- GUPTA S, BANFIELD C, AFFRIME M, MARBURY T, PADHI D, GLUE P (2002). Oral bioavailability of desloratadine is unaffected by food. *Clin Pharmacokinet.* **41** Suppl 1:7-12.
- HANF G, SCHIERHORN K, BRUNNEE T, NOGA O, VERGES D, KUNKEL G (2000). Substance P induced histamine release from nasal mucosa of subjects with and without allergic rhinitis. *Inflamm Res.* **49**(10):520-3.
- HANIF J, JAWAD SS, ECCLES R (2000). The nasal cycle in health and disease. *Clin Otolaryngol.* **25**(6):461-7.
- HARTMAN CT Jr., GLOVSKY MM (1981). Complement activation requirements for histamine release from human leukocytes: influence of purified C3ahu and C5ahu on histamine release. *Int Arch Allergy Appl Immunol.* **66**(3): 274-81.
- HAVAS TE, COLE P, PARKER L, OPRYSK D, AYIOMAMITIS A (1986). The effects of combined H<sub>1</sub> and H<sub>2</sub> histamine antagonists on alterations in nasal airflow resistance induced by topical histamine provocation. *J Allergy Clin Immunol.* **78**(5 Pt 1):856-60.
- HEY JA, ASLANIAN R, BOLSER DC, CHAPMAN RW, EGAN RW, RIZZO CA, SHIH NY, FERNANDEZ X, MCLEOD RL, WEST R, KREUTNER W (1998). Studies on the pharmacology of the novel histamine H<sub>3</sub> receptor agonist Sch 50971. *Arzneimittelforschung.* **48**(9):881-8.
- HILBERG O, GRYMER LF, PEDERSEN OF (1995). Nasal histamine challenge in nonallergic and allergic subjects evaluated by acoustic rhinometry. *Allergy.* **50**(2):166-73.
- HILBERG O, JACKSON AC, SWIFT DL, PEDERSEN OF (1989). Acoustic rhinometry: evaluation of nasal cavity geometry by acoustic reflection. *J Appl Physiol.* **66**(1):295-303.
- HIRASAWA A, HORIE K, TANAKA T, TAKAGAKI K, MURAI M, YANO J, TSUJIMOTO G (1993). Cloning, functional expression and tissue distribution of human cDNA for the alpha 1C-adrenergic receptor. *Biochem Biophys Res Commun.* **195**(2):902-9.
- HIRATA N, TAKEUCHI K, UKAI K, JIN C, YOSHIDA T, SAKAKURA Y (1999a). Expression and localization of histamine H<sub>2</sub> receptor messenger RNA in human nasal mucosa. *J Allergy Clin Immunol.* **103**(5 Pt 1):944-9.
- HIRATA N, TAKEUCHI K, UKAI K, SAKAKURA Y (1999b). Expression of histidine decarboxylase messenger RNA and histamine N-methyltransferase messenger RNA in nasal allergy. *Clin Exp Allergy.* **29**(1):76-83.
- HIRSCHOWITZ BI, DANILEWITZ M, MOLINA E (1982). Inhibition of basal acid, chloride, and pepsin secretion in duodenal ulcer by graded doses of ranitidine and atropine with studies of pharmacokinetics of ranitidine. *Gastroenterology.* **82**(6):1314-26.
- HOCHBAN W, ALTHOFF H, ZIEGLER A (1999). Nasal decongestion with imidazoline derivatives: acoustic rhinometry measurements. *Eur J Clin Pharmacol.* **55**(1):7-12.

- HOLGATE ST, BROIDE D (2003). New targets for allergic rhinitis - a disease of civilization. *Nat Rev Drug Discov.* **2**(11):903-15.
- HOLLOWAY RH, KULJIAN B, ESHELMAN F, MCCALLUM RW (1984). Effects of ranitidine and of cimetidine on pentagastrin-stimulated gastric acid secretion. *Clin Pharmacol Ther.* **35**(2):203-7.
- HOLMBERG K, BAKE B, PIPKORN U (1988). Nasal mucosal blood flow after intranasal allergen challenge. *J Allergy Clin Immunol.* **81**(3):541-7.
- HOLMBERG K, BAKE B, PIPKORN U (1989a). Reflex activation in allergen-induced nasal mucosal vascular reactions. *Acta Otolaryngol.* **108**(1-2):130-5.
- HOLMBERG K, BAKE B, PIPKORN U (1989b). The effect of topical nasal anaesthesia on allergen-induced symptoms, obstruction and blood flow. *Clin Exp Allergy.* **19**(4):443-7.
- HOLMBERG K, BAKE B, PIPKORN U (1990). Vascular effects of topically applied bradykinin on the human nasal mucosa. *Eur J Pharmacol.* **175**(1):35-41.
- HOLMBERG K, PIPKORN U, BAKE B, BLYCHERT LO (1989c). Effects of topical treatment with H<sub>1</sub> and H<sub>2</sub> antagonists on clinical symptoms and nasal vascular reactions in patients with allergic rhinitis. *Allergy.* **44**(4):281-7.
- HONING ML, SMITS P, MORRISON PJ, RABELINK TJ (2000). Bradykinin-induced vasodilation of human forearm resistance vessels is primarily mediated by endothelium-dependent hyperpolarization. *Hypertension.* **35**(6):1314-8.
- HORAK F, STUBNER U, ZIEGLMAYER R, HARRIS A (2002). Effect of desloratadine versus placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. *J Allergy Clin Immunol.* **109**(6):956-61.
- HORVATH G, SUTTO Z, TORBATI A, CONNER GE, SALATHE M, WANNER A (2003). Norepinephrine transport by the extraneuronal monoamine transporter in human bronchial arterial smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol.* **285**(4):L829-37.
- HOWARTH PH (1995). The cellular basis for allergic rhinitis. *Allergy.* **50**(23 Suppl):6-10.
- HOWARTH PH, SALAGEAN M, DOKIC D (2000). Allergic rhinitis: not purely a histamine-related disease. *Allergy.* **55** (Suppl 64):7-16.
- HUANG SM, ATHANIKAR NK, SRIDHAR K, HUANG YC, CHIOU WL (1982). Pharmacokinetics of chlorpheniramine after intravenous and oral administration in normal adults. *Eur J Clin Pharmacol.* **22**(4):359-65.
- HUGHES IE, SMITH JA (1978). The stability of noradrenaline in physiological saline solutions. *J Pharm Pharmacol.* **30**(2):124-6.
- ICHIMURA K, CHOW MJ (1988a). Postjunctional alpha<sub>2</sub>-adrenoceptors in blood vessels of human nasal mucosa. *Arch Otorhinolaryngol.* **245**(2):127-31.
- ICHIMURA K, MINEDA H, SEKI A (1988b). Vascular effects of neuropeptides on nasal mucosa. *Ann Otol Rhinol Laryngol.* **97**(3 Pt 1):289-93.
- ICHINOSE M, BARNES PJ (1989). Inhibitory histamine H<sub>3</sub>-receptors on cholinergic nerves in human airways. *Eur J Pharmacol.* **163**(2-3):383-6.

- IKEDA H, KUBO N, NAKAMURA A, HARADA N, MINAMINO M, YAMASHITA T (1997). Histamine-induced calcium released from cultured human mucosal microvascular endothelial cells from nasal inferior turbinate. *Acta Otolaryngol.* **117**(6):864-70.
- IMADA M, NONAKA S, KOBAYASHI Y, IWAMOTO J (2002). Functional roles of nasal nitric oxide in nasal patency and mucociliary function. *Acta Otolaryngol.* **122**(5):513-9.
- IMAMURA T, DUBIN A, MOORE W, TANAKA R, TRAVIS J (1996). Induction of vascular permeability enhancement by human tryptase: dependence on activation of prekallikrein and direct release of bradykinin from kininogens. *Lab Invest.* **74**(5):861-70.
- IRIFUNE M, OGINO S, HARADA T, MATSUNAGA T (1993). Acute and chronic effects of unilateral cervical sympathectomy on nasal allergy-like symptoms induced by toluene diisocyanate in guinea pigs. *Acta Otolaryngol Suppl.* **501**:97-9.
- IRIYOSHI N, TAKEUCHI K, YUTA A, UKAI K, SAKAKURA Y (1996). Increased expression of histamine H<sub>1</sub> receptor mRNA in allergic rhinitis. *Clin Exp Allergy.* **26**(4):379-85.
- ISHIBE T, KUBO N, KUMAZAWA H, YAMASHITA T, KAMAZAWA T (1985). Histamine H<sub>1</sub> receptors and affinity analyses in human nasal mucosa in cases of nasal allergy. *Ann Otol Rhinol Laryngol.* **94**(2 Pt 1):186-90.
- ISHIKAWA S, SPERELAKIS N (1987). A novel class (H<sub>3</sub>) of histamine receptors on perivascular nerve terminals. *Nature.* **327**(6118):158-60.
- JACKSON RT, STEELE RW (1985). The inhibition of norepinephrine release in nasal mucosa by acetylcholine. *Rhinology.* **23**(1):59-64.
- JANSEN FP, MOCHIZUKI T, YAMAMOTO Y, TIMMERMAN H, YAMATODANI A (1998). In vivo modulation of rat hypothalamic histamine release by the histamine H<sub>3</sub> receptor ligands, imipip and clobenpropit. Effects of intrahypothalamic and peripheral application. *Eur J Pharmacol.* **362**(2-3):149-55.
- JOHANNSEN V, MAUNE S, WERNER JA, RUDERT H, ZIEGLER A (1997). Alpha<sub>1</sub>-receptors at pre-capillary resistance vessels of the human nasal mucosa. *Rhinology.* **35**(4):161-5.
- JOHNSON DA, HRICIK JG (1993). The pharmacology of alpha-adrenergic decongestants. *Pharmacotherapy.* **13**(6 Pt 2):110S-115S; discussion 143S-146S.
- JOHNSTON SL, PRICE JN, LAU LC, WALLS AF, WALTERS C, FEATHER IH, HOLGATE ST, HOWARTH PH (1993). The effect of local hyperthermia on allergen-induced nasal congestion and mediator release. *J Allergy Clin Immunol.* **92**(6):850-6.
- JOSE PJ, FORREST MJ, WILLIAMS TJ (1981). Human C5a des Arg increases vascular permeability. *J Immunol.* **127**(6): 2376-80.
- JULIUSSON S, BENDE M (1996). Effect of systemically administered H<sub>1</sub>- and H<sub>2</sub>-receptor antagonists on nasal blood flow as measured with laser Doppler flowmetry in a provoked allergic reaction. *Rhinology.* **34**(1):24-7.
- JUSTICE JP, SHIBATA Y, SUR S, MUSTAFA J, FAN M, VAN SCOTT MR (2001). IL-10 gene knockout attenuates allergen-induced airway hyperresponsiveness in C57BL/6 mice. *Am J Physiol Lung Cell Mol Physiol.* **280**(2):L363-8.
- JUTO JE, LUNDBERG C (1984). Nasal mucosa reaction, catecholamines and lactate during physical exercise. *Acta Otolaryngol.* **98**(5-6):533-42.

- KAISER HB, FINDLAY SR, GEORGITIS JW, GROSSMAN J, RATNER PH, TINKELMAN DG, ROSZKO P, ZEGARELLI E, WOOD CC (1995). Long-term treatment of perennial allergic rhinitis with ipratropium bromide nasal spray 0.06%. *J Allergy Clin Immunol.* **95**(5 Pt 2):1128-32.
- KALINER MA (1994). The mediators of the early and late phases of allergic rhinitis. *Adv Prostaglandin Thromboxane Leukot Res.* **22**:271-8.
- KATO M, NISHIDA A, AGA Y, KITA J, KUDO Y, NARITA H, ENDO T (1997). Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate. *Arzneimittelforschung.* **47**(10):1116-24.
- KAVANAGH GM, SABROE RA, GREAVES MW, ARCHER CB (1998). The intradermal effects of the H<sub>3</sub> receptor agonist R alpha methylhistamine in human skin. *Br J Dermatol.* **138**(4):622-6.
- KAWARAI M, KOSS MC (2001). Sympathetic control of nasal blood flow in the rat mediated by alpha<sub>1</sub>-adrenoceptors. *Eur J Pharmacol.* **413**(2-3):255-62.
- KEMP A, BJORKSTEN B (2003). Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatr Allergy Immunol.* **14**(2):74-80.
- KENNEDY B, ZIEGLER MG, SHANNAHOFF-KHALSA DS (1986). Alternating lateralization of plasma catecholamines and nasal patency in humans. *Life Sci.* **38**(13):1203-14.
- KILLACKEY JJ, JOHNSTON MG, MOVAT HZ (1986). Increased permeability of microcarrier-cultured endothelial monolayers in response to histamine and thrombin. A model for the in vitro study of increased vasopermeability. *Am J Pathol.* **122**(1):50-61.
- KIRBY RS (1999). Clinical pharmacology of alpha<sub>1</sub>-adrenoceptor antagonists. *Eur Urol.* **36 Suppl 1**:48-53; discussion 65.
- KIRKEGAARD J, SECHER C, BORUM P, MYGIND N (1983). Inhibition of histamine-induced nasal symptoms by the H<sub>1</sub> antihistamine chlorpheniramine maleate: demonstration of topical effect. *Br J Dis Chest.* **77**(2):113-22.
- KITA H, ABU-GHAZALEH RI, SUR S, GLEICH GJ (1995). Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. *J Immunol.* **154**(9):4749-58.
- KLEINJAN A, HOLM AF, DIJKSTRA MD, BOKS SS, SEVERIJNEN L, MULDER PG FOKKENS WJ (2000). Preventive treatment of intranasal fluticasone propionate reduces cytokine mRNA expressing cells before and during a single nasal allergen provocation. *Clin Exp Allergy.* **30**(10):1476-85.
- KLIMEK L, RESKE-KUNZ AB, SALOGA J (1999). Theories on the mode of action of desensitization. *Wien Med Wochenschr.* **149**(14-15):415-20.
- KLOCKENBUSCH W, BRAUN MS, SCHRODER H, HECKENBERGER RE, STROBACH H, SCHROR K (1992). Prostacyclin rather than nitric oxide lowers human umbilical artery tone in vitro. *Eur J Obstet Gynecol Reprod Biol.* **47**(2):109-15.
- KNAPP HR (1990). Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. *N Engl J Med.* **323**(25):1745-8.
- KNAPP HR, MURRAY JJ (1994). Leukotrienes as mediators of nasal inflammation. *Adv Prostaglandin Thromboxane Leukot Res.* **22**:279-88.

- KNIGHT SE, MCISAAC RL, RENNIE CD (1980). The effect of histamine and histamine antagonists on gastric acid secretion and mucosal blood flow in man. *Br J Surg.* **67**(4):266-8.
- KNOX JH, JURAND J (1976). Separation of catecholamines and their metabolites by adsorption, ion-pair and soap chromatography. *J Chromatogr.* **125**(1):89-101.
- KOBAYASHI M, UKAI K, TATEMATSU M, MATSUURA T, SAKAKURA Y (2001). Late phase responses after nasal challenges with allergen and histamine in asthmatic children with perennial nasal allergy. *Auris Nasus Larynx.* **28**(4):305-10.
- KOHNO S, NAKAO S, OGAWA K, YAMAMURA H, NABE T, OHATA K (1994). Possible participation of histamine H<sub>3</sub>-receptors in the regulation of anaphylactic histamine release from isolated rat peritoneal mast cells. *Jpn J Pharmacol.* **66**(2):173-80.
- KONNO A, NUMATA T, TERADA N, HANAZAWA T, NAGATA H, MOTOSUGI H (1996). Role of substance P in the vascular response of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol.* **105**(8):648-53.
- KONNO A, TERADA N, OKAMOTO Y (1987). Responses of resistance and capacitance vessels in human nasal mucosa to beta-receptor agonists. *J Otorhinolaryngol Relat Spec.* **49**(4):206-13.
- KONNO A, TOGAWA K (1979). Role of the vidian nerve in nasal allergy. *Ann Otol Rhinol Laryngol.* **88**(2 Pt 1):258-66.
- KONNO A, TOGAWA K, ITASAKA Y (1982). Neurophysiological mechanism of shrinkage of nasal mucosa induced by exercise. *Auris Nasus Larynx.* **9**(2):81-90.
- KOPIN IJ (1972). Metabolic degradation of catecholamines. The relative importance of different pathways under physiological conditions and after administration of drugs. *Handb Exp Pharm.* **33**(Chapter 8):270-82.
- KORNEL L (1993). The role of vascular steroid receptors in the control of vascular contractility and peripheral vascular resistance. *J Steroid Biochem Mol Biol.* **45**(1-3):195-203.
- KOVALHUK LC, ROSARIO NA, CARVALHO A (2001). Inflammatory mediators, cell counts in nasal lavage and computed tomography of the paranasal sinuses in atopic children. *J Pediatr (Rio J).* **77**(4):271-8.
- KOZIK A, MOORE RB, POTEMPA J, IMAMURA T, RAPALA-KOZIK M, TRAVIS J (1998). A novel mechanism for bradykinin production at inflammatory sites. Diverse effects of a mixture of neutrophil elastase and mast cell tryptase versus tissue and plasma kallikreins on native and oxidized kininogens. *J Biol Chem.* **273**(50):33224-9.
- KRAAN J, VINK-KLOOSTER H, POSTMA DS (2001). The NK<sub>2</sub> receptor antagonist SR 48968C does not improve adenosine hyperresponsiveness and airway obstruction in allergic asthma. *Clin Exp Allergy.* **31**(2):274-8.
- KRISTIANSEN AB, HEYERAAS KJ, KIRKEBO A (1993). Increased pressure in venous sinusoids during decongestion of rat nasal mucosa induced by adrenergic agonists. *Acta Physiol Scand.* **147**(2):151-61.
- KUBO N, KOZUKI K, TSUJI H, YAMASHITA T, KUMAZAWA T (1989a). Norepinephrine content in guinea-pig nasal mucosa response to histamine and methacholine provocation. *Acta Otolaryngol Suppl.* **463**:39-43.

- KUBO N, TSUJI H, MINAMI T, YAMASHITA T, KUMAZAWA T (1989b). Histamine and acetylcholine regulation of sympathetic nerve activity in guinea-pig nasal mucosa. *Acta Otolaryngol Suppl.* **463**:29-38.
- KUNKEL G, MYGIND N, OKUDA M, VAN CAUWENBERGE P (1987). Sodium cromoglycate. *Allergic and Vasomotor Rhinitis: Clinical Aspects.* 159-164.
- LACROIX JS, CORREIA F, FATHI M, GROUZMANN E (1997). Post-exercise nasal vasoconstriction and hyporeactivity: possible involvement of neuropeptide Y. *Acta Otolaryngol.* **117**(4):609-13.
- LACROIX JS, LUNDBERG JM (1989). Adrenergic and neuropeptide Y supersensitivity in denervated nasal mucosa vasculature of the pig. *Eur J Pharmacol.* **169**(1):125-36.
- LACROIX JS, STJARNE P, ANGGARD A, LUNDBERG JM (1988a). Sympathetic vascular control of the pig nasal mucosa (2): Reserpine-resistant, non-adrenergic nervous responses in relation to neuropeptide Y and ATP. *Acta Physiol Scand.* **133**(2):183-97.
- LACROIX JS, STJARNE P, ANGGARD A, LUNDBERG JM (1988b). Sympathetic vascular control of the pig nasal mucosa: (I). Increased resistance and capacitance vessel responses upon stimulation with irregular bursts compared to continuous impulses. *Acta Physiol Scand.* **132**(1):83-90.
- LACROIX JS, ULMAN LG, POTTER EK (1994a). Sympathetic and parasympathetic interaction in vascular control of the nasal mucosa in anaesthetized cats. *J Physiol.* **480**(Pt 2):325-31.
- LACROIX JS, ULMAN LG, POTTER EK (1994b). The role of ATP in non-adrenergic sympathetic vascular control of the nasal mucosa in anaesthetized cats and dogs. *J Physiol.* **476**(3):429-35.
- LARSSON M, FORSMAN A, HALLGREN J (1987). HPLC assays of noradrenaline and dopamine in CSF samples from drug-free subjects. *Methods Find Exp Clin Pharmacol.* **9**(10):669-72.
- LE GRAS MD, SEIFERT B, CASIRO O (1990). Neonatal nasal obstruction associated with methyldopa treatment during pregnancy. *Am J Dis Child.* **144**(2):143-4.
- LEVER R, HOULT JR, PAGE CP (2000). The effects of heparin and related molecules upon the adhesion of human polymorphonuclear leucocytes to vascular endothelium in vitro. *Br J Pharmacol.* **129**(3):533-40.
- LI X, DU B, GUO X (1997). Relationship between nasal airflow sensation and nasal patency. *Zhonghua Er Bi Yan Hou Ke Za Zhi.* **32**(2):109-11.
- LIU AH, MURPHY JR (2003). Hygiene hypothesis: fact or fiction? *J Allergy Clin Immunol.* **111**(3):471-8.
- LLONA I, GALLEGUILLOS X, BELMAR J, HUIDOBRO-TORO JP (1991). Bradykinin modulates the release of noradrenaline from vas deferens nerve terminals. *Life Sci.* **48**(26):2585-92.
- LLOYD CM, RANKIN SM (2003). Chemokines in allergic airway disease. *Curr Opin in Pharmacol.* **3**(4):443-8.
- LORIN MI, GAERLAN PF, MANDEL ID (1972). Quantitative composition of nasal secretions in normal subjects. *J Lab Clin Med.* **80**(2):275-81.

- LOVENBERG TW, ROLAND BL, WILSON SJ, JIANG X, PYATI J, HUVAR A, JACKSON MR, ERLANDER MG (1999). Cloning and functional expression of the human histamine H<sub>3</sub> receptor. *Mol Pharmacol.* **55**(6):1101-7.
- LOWMAN MA, REES PH, BENYON RC, CHURCH MK (1988). Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. *J Allergy Clin Immunol.* **81**(3):590-7.
- LU K, QI H, WANG W (1997). The relationship between interleukin-8 and airway hyperresponsiveness. *Zhonghua Jie He He Hu Xi Za Zhi.* **20**(5):280-2.
- LUNDBERG JM, MODIN A (1995). Inhibition of sympathetic vasoconstriction in pigs in vivo by the neuropeptide Y-Y1 receptor antagonist BIBP 3226. *Br J Pharmacol.* **116**(7):2971-82.
- LUNG MA, PHIPPS RJ, WANG JC, WIDDICOMBE JG (1984). Control of nasal vasculature and airflow resistance in the dog. *J Physiol.* **349**:535-51.
- LUO HY, WEAD WB, YANG S, WILSON MA, HARRIS PD (1995). Nitric oxide mediates C5a-induced vasodilation in the small intestine. *Microcirculation.* **2**(1):53-61.
- MALIS DD, RIST B, NICOUCAR K, BECK-SICKINGER AG, MOREL DR, LACROIX JS (2001). Modulatory effect of two novel CGRP receptor antagonists on nasal vasodilatory responses to exogenous CGRP, capsaicin, bradykinin and histamine in anaesthetised pigs. *Regul Pept.* **101**(1-3):101-8.
- MALM L (1977). Sympathetic influence on the nasal mucosa. *Acta Otolaryngol.* **83**(1-2):20-1.
- MALM L (1997). Measurement of nasal patency. *Allergy.* **52**(40 Suppl):19-23.
- MALM L, OKUDA M, DIEGES PH (1987). Direct and reflex actions of biochemical mediators. *Allergic and Vasomotor Rhinitis: Pathophysiological Aspects.* 214-219.
- MANCAMA D, ARRANZ MJ, MUNRO J, OSBORNE S, MAKOFF A, COLLIER D, KERWIN R (2002). Investigation of promoter variants of the histamine 1 and 2 receptors in schizophrenia and clozapine response. *Neurosci Lett.* **333**(3):207-11.
- MARCUCCI F, SENSI LG, MIGALI E, CONIGLIO G (2001). Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. *Allergy.* **56**(3):231-6.
- MARGOLIS J (1958). Activation of plasma by contact with glass: evidence for a common reaction which releases plasma kinin and initiates coagulation. *J Physiol.* **144**(1):1-22.
- MATSUBARA S, KIKKAWA H, KAMINUMA O, IKEZAWA K (1997). Angiotensin-converting enzyme inhibitors can potentiate ozone-induced airway hyperresponsiveness. *Eur J Pharmacol.* **337**(2-3):259-65.
- MAYNARD ML, ERNST ME (2001). Leukotriene receptor antagonists in the treatment of allergic rhinitis. *Ann Pharmacother.* **35**(10):1274-7.
- MCLEAN JA, BACON JR, MATHEWS KP, BANAS J, CAPATI D, BAYNE NK (1983). Effects of aspirin on nasal responses in atopic subjects. *J Allergy Clin Immunol.* **72**(2):187-92.



- MCLEOD RL, ERICKSON CH, MINGO GG, HEY JA (2001). Intranasal application of the  $\alpha_2$ -adrenoceptor agonist BHT-920 produces decongestion in the cat. *Am J Rhinol.* **15**(6):407-15.
- MCLEOD RL, MINGO GG, HERCZKU C, CORBOZ MR, RAMOS SI, DEGENNARO-CULVER F, PEDERSEN O, HEY JA (1999a). Changes in nasal resistance and nasal geometry using pressure and acoustic rhinometry in a feline model of nasal congestion. *Am J Rhinol.* **13**(5):375-83.
- MCLEOD RL, MINGO GG, HERCZKU C, DEGENNARO-CULVER F, KREUTNER W, EGAN RW, HEY JA (1999b). Combined histamine  $H_1$  and  $H_3$  receptor blockade produces nasal decongestion in an experimental model of nasal congestion. *Am J Rhinol.* **13**(5):391-9.
- MCLEOD RL, RIZZO CA, WEST RE Jr., ASLANIAN R, MCCORMICK K, BRYANT M, HSIEH Y, KORFMACHER W, MINGO GG, VARTY L, WILLIAMS SM, SHIH NY, EGAN RW, HEY JA (2003). Pharmacological characterization of the novel histamine  $H_3$ -receptor antagonist N-(3,5-dichlorophenyl)-N'-[[4-(1H-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687). *J Pharmacol Exp Ther.* **305**(3):1037-44.
- MCNEIL JJ, MIHALY GW, ANDERSON A, MARSHALL AW, SMALLWOOD RA, LOUIS WJ (1981). Pharmacokinetics of the  $H_2$ -receptor antagonist ranitidine in man. *Br J Clin Pharmacol.* **12**(3):411-5.
- MIADONNA A, COTTINI M, MILAZZO N, TOSI D, DANZIG M, TEDESCHI A (1998). In vivo and ex vivo inhibitory effects of loratadine on histamine release in patients with allergic rhinitis. *Allergy.* **53**(12):1183-8.
- MIADONNA A, MILAZZO N, GIBELLI S, SALMASO C, LORINI M, TEDESCHI A (1999). Nasal response to a single antigen challenge in patients with allergic rhinitis - inflammatory cell recruitment persists up to 48 hours. *Clin Exp Allergy.* **29**(7):941-9.
- MIADONNA A, TEDESCHI A, LEGGIERI E, LORINI M, FOLCO G, SALA A, QUALIZZA R, FROLDI M, ZANUSSI C (1987). Behavior and clinical relevance of histamine and leukotrienes C4 and B4 in grass pollen-induced rhinitis. *Am Rev Respir Dis.* **136**(2):357-62.
- MIADONNA A, TEDESCHI A, LEGGIERI E, LORINI M, QUALIZZA R, FROLDI M, ZANUSSI C (1988). Activity of substance P on human skin and nasal airways. *Ann Allergy.* **61**(3):220-3.
- MILLS JG, WOOD JR (1989). The pharmacology of histamine  $H_2$ -receptor antagonists. *Methods Find Exp Clin Pharmacol.* **11** (Suppl 1):87-95.
- MITA H, ISHII T, YAMADA T, AKIYAMA K, SHIDA T (1993). Further characterization of dispersed human sinus mast cells. *Life Sci.* **53**(10):775-82.
- MOLDERINGS GJ, WEISSENBORN G, SCHLICKER E, LIKUNGU J, GOTHERT M (1992). Inhibition of noradrenaline release from the sympathetic nerves of the human saphenous vein by presynaptic histamine  $H_3$  receptors. *Naunyn Schmiedebergs Arch Pharmacol.* **346**(1):46-50.
- MOSER M (1958). Phenoxybenzamine. *The Practitioner.* **180**:609-16.
- MOSIMANN BL, WHITE MV, HOHMAN RJ, GOLDRICH MS, KAULBACH HC, KALINER MA (1993). Substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide increase in nasal secretions after allergen challenge in atopic patients. *J Allergy Clin Immunol.* **92**(1 Pt 1):95-104.

- MOTOJIMA S, FRIGAS E, LOEGERING DA, GLEICH GJ (1989). Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis*. **139**(3):801-5.
- MOUSLI M, BRONNER C, BUEB JL, TSCHIRHART E, GIES JP, LANDRY Y (1989). Activation of rat peritoneal mast cells by substance P and mastoparan. *J Pharmacol Exp Ther*. **250**(1):329-35.
- MOUSLI M, BUEB JL, ROUOT B, LANDRY Y, BRONNER C (1991). G-proteins as targets for non-immunological histamine releasers. *Agents Actions*. **33**(1-2):81-3.
- MOYER JH, CAPLOVITZ C (1953). The Clinical results of oral and parenteral administration of 2-(N'p-tolyl-N'm-hydroxyphenylaminomethyl) imidazoline hydrochloride (Regitine) in the treatment of hypertension and an evaluation of the cerebral hemodynamic effects. *Am Heart J*. **45**:602-10.
- MULLINS RJ, OLSON LG, SUTHERLAND DC (1989). Nasal histamine challenges in symptomatic allergic rhinitis. *J Allergy Clin Immunol*. **83**(5):955-9.
- MURR G (1976). Influence of reserpine on the nasal mucous membrane. *Laryngol Rhinol Otol (Stuttg)*. **55**(3):178-85.
- MYGIND N (1982). Mediators of nasal allergy. *J Allergy Clin Immunol*. **70**(3):149-59.
- MYGIND N, SECHER C, KIRKEGAARD J (1983). Role of histamine and antihistamines in the nose. *Eur J Respir Dis Suppl*. **128**(Pt 1):16-20.
- NACLERIO R, SOLOMON W (1997). Rhinitis and inhalant allergens. *JAMA*. **278**(22):1842-8.
- NACLERIO RM (1990). The role of histamine in allergic rhinitis. *J Allergy Clin Immunol*. **86**(4 Pt 2):628-32.
- NACLERIO RM (1997). Pathophysiology of perennial allergic rhinitis. *Allergy*. **52**(36 Suppl):7-13.
- NACLERIO RM, MEIER HL, KAGEY-SOBOTKA A, ADKINSON NF Jr., MEYERS DA, NORMAN PS, LICHTENSTEIN LM (1983). Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis*. **128**(4):597-602.
- NACLERIO RM, PROUD D, TOGIAS AG, ADKINSON NF, MEYERS DA, KAGEY-SOBOTKA A, PLAUT M, NORMAN PS, LICHTENSTEIN LM (1985). Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med*. **313**(2):65-70.
- NAGAI M, NAGAI T, TONO T (1984). Scanning electron microscopy of nasal arterioles effected by vasoactive agents. *Arch Otorhinolaryngol*. **241**(1):45-9.
- NAITO K, KOMORI M, KONDO Y, TAKEUCHI M, IWATA S (1997). The effect of L-menthol stimulation of the major palatine nerve on subjective and objective nasal patency. *Auris Nasus Larynx*. **24**(2):159-62.
- NAKAMURA T, ITADANI H, HIDAKA Y, OHTA M, TANAKA K (2000). Molecular cloning and characterization of a new human histamine receptor, HH<sub>4</sub>R. *Biochem Biophys Res Commun*. **279**(2):615-20.
- NAYAK AS, SCHENKEL E (2001). Desloratadine reduces nasal congestion in patients with intermittent allergic rhinitis. *Allergy*. **56**(11):1077-80.

- NIELSEN LP, BJERKE T, CHRISTENSEN MB, SKAMLING M, PETERSON CG, MYGIND N, DAHL R (1998). Eosinophil markers in seasonal allergic rhinitis. Intranasal fluticasone propionate inhibits local and systemic increases during the pollen season. *Allergy*. **53**(8):778-85.
- NOURI-ARIA KT, O'BRIEN F, NOBLE W, JACOBSON MR, RAJAKULASINGAM K, DURHAM SR (2000). Cytokine expression during allergen-induced late nasal responses: IL-4 and IL-5 mRNA is expressed early (at 6 h) predominantly by eosinophils. *Clin Exp Allergy*. **30**(12):1709-16.
- OKAYAMA M, BARANIUK JN, HAUSFELD JN, MERIDA M, KALINER MA (1992). Characterization and autoradiographic localization of histamine H<sub>1</sub> receptors in human nasal turbinates. *J Allergy Clin Immunol*. **89**(6):1144-50.
- OKAYAMA M, MULLOL J, BARANIUK JN, HAUSFELD JN, FELDMAN B, MERIDA M, SHELHAMER JH, KALINER MA (1993). Muscarinic receptor subtypes in human nasal mucosa: characterization, autoradiographic localization, and function in vitro. *Am J Respir Cell Mol Biol*. **8**(2):176-87.
- OLSSON P (1986). A comparison between the <sup>133</sup>Xe washout and laser Doppler techniques for estimation of nasal mucosal blood flow in humans. *Acta Otolaryngol*. **102**(1-2):106-12.
- ORTIZ JL, LABAT C, NOREL X, GORENNE I, VERLEY J, BRINK C (1992). Histamine receptors on human isolated pulmonary arterial muscle preparations: effects of endothelial cell removal and nitric oxide inhibitors. *J Pharmacol Exp Ther*. **260**(2):762-7.
- OTSUKA H, INABA M, FUJIKURA T, KUNITOMO M (1995). Histochemical and functional characteristics of metachromatic cells in the nasal epithelium in allergic rhinitis: studies of nasal scrapings and their dispersed cells. *J Allergy Clin Immunol*. **96**(4):528-36.
- OTTOSSON A, JANSEN I, EDVINSSON L (1989). Pharmacological characterization of histamine receptors in the human temporal artery. *Br J Clin Pharmacol*. **27**(2):139-45.
- OVERLACK A, MULLER B, SCHMIDT L, SCHEID ML, MULLER M, STUMPE KO (1992). Airway responsiveness and cough induced by angiotensin converting enzyme inhibition. *J Hum Hypertens*. **6**(5):387-92.
- OWEN DA, POY E, WOODWARD DF, DANIEL D (1980). Evaluation of the role of Histamine H<sub>1</sub>- and H<sub>2</sub>-receptors in cutaneous inflammation in the guinea-pig produced by histamine and mast cell degranulation. *Br J Pharmacol*. **69**(4):615-23.
- PAQUAY JB, HOEN PA, VOSS HP, BAST A, TIMMERMAN H, HAENEN GR (1999). Nitric oxide synthase inhibition by dimaprit and dimaprit analogues. *Br J Pharmacol*. **127**(2):331-4.
- PARSONS ME, OWEN DA, GANELLIN CR, DURANT GJ (1977). Dimaprit -(S-[3-(N,N-dimethylamino)propyl]isothiourea) - a highly specific histamine H<sub>2</sub> -receptor agonist. Part 1. *Pharmacology. Agents Actions*. **7**(1):31-7.
- PASTORELLO EA, RIARIO-SFORZA GG, INCORVAIA C, SEGALA M, FUMAGALLI M, GANDINI R (1994). Comparison of rhinomanometry, symptom score, and inflammatory cell counts in assessing the nasal late-phase reaction to allergen challenge. *J Allergy Clin Immunol*. **93**(1 Pt 1):85-92.
- PAUL W, DOUGLAS GJ, LAWRENCE L, KHAWAJA AM, PEREZ AC, SCHACHTER M, PAGE CP (1994). Cutaneous permeability responses to bradykinin and histamine in the guinea-pig: possible differences in their mechanism of action. *Br J Pharmacol*. **111**(1):159-64.

- PAWLAK D, MALINOWSKA B, WOLLNY T, GODLEWSKI G, BUCZKO W. (1996). Lack of the specific influence of histamine and histamine H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptor ligands on the serotonin uptake and release in rat blood platelets. *Pol J Pharmacol.* **48**(6):615-20.
- PIETRUCK C, GROND S, XIE GX, PALMER PP (2003). Local anesthetics differentially inhibit sympathetic neuron-mediated and C fiber-mediated synovial neurogenic plasma extravasation. *Anesth Analg.* **96**(5):1397-402.
- PIPKORN U, GRANERUS G, PROUD D, KAGEY-SOBOTKA A, NORMAN PS, LICHTENSTEIN LM, NACLERIO RM (1987). The effect of a histamine synthesis inhibitor on the immediate nasal allergic reaction. *Allergy.* **42**(7):496-501.
- PIRILA T, NUUTINEN J (1998). Acoustic rhinometry, rhinomanometry and the amount of nasal secretion in the clinical monitoring of the nasal provocation test. *Clin Exp Allergy.* **28**(4):468-77.
- POLWIN W, MCDONALD FM, BRINKMANN C, HIRCHE H, ADDICKS K (1987). Effects of lidocaine on catecholamine release in the ischemic rat heart. *J Cardiovasc Pharmacol.* **9**(1):6-11.
- POWELL JR, BRODY MJ (1976a). Identification and blockade of vascular H<sub>2</sub> receptors. *Fed Proc.* **35**(8):1935-41.
- POWELL JR, BRODY MJ (1976b). Participation of H<sub>1</sub> and H<sub>2</sub> histamine receptors in physiological vasodilator responses. *Am J Physiol.* **231**(4):1002-9.
- PREUSS JM, PAGE CP (2000). Effect of heparin on antigen-induced airway responses and pulmonary leukocyte accumulation in neonatally immunized rabbits. *Br J Pharmacol.* **129**(8):1585-96.
- PRONK-ADMIRAAL CJ, BARTELS PC (2001). Total amount of ECP per eosinophil as indicator for the activity state of eosinophils. *Scand J Clin Lab Invest.* **61**(6):453-7.
- PROUD D, BAUMGARTEN CR, NACLERIO RM, WARD PE (1987). Kinin metabolism in human nasal secretions during experimentally induced allergic rhinitis. *J Immunol.* **138**(2):428-34.
- PROUD D, KAPLAN AP (1988a). Kinin formation: mechanisms and role in inflammatory disorders. *Annu Rev Immunol.* **6**:49-83.
- PROUD D, NACLERIO RM, MEYERS DA, KAGEY-SOBOTKA A, LICHTENSTEIN LM, VALENTINE MD (1990). Effects of a single-dose pretreatment with captopril on the immediate response to nasal challenge with allergen. *Int Arch Allergy Appl Immunol.* **93**(2-3):165-70.
- PROUD D, REYNOLDS CJ, LACAPRA S, KAGEY-SOBOTKA A, LICHTENSTEIN LM, NACLERIO RM (1988b). Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis.* **137**(3):613-6.
- PROUD D, TOGIAS A, NACLERIO RM, CRUSH SA, NORMAN PS, LICHTENSTEIN LM (1983). Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J Clin Invest.* **72**(5):1678-85.
- PULLERITS T, PRAKS L, RISTIOJA V, LOTVALL J (2002). Comparison of a nasal glucocorticoid, antileukotriene, and a combination of antileukotriene and antihistamine in the treatment of seasonal allergic rhinitis. *J Allergy Clin Immunol.* **109**(6):949-55.

- RAJAKULASINGAM K, POLOSA R, LAU LC, CHURCH MK, HOLGATE ST, HOWARTH PH (1992). Nasal effects of bradykinin and capsaicin: influence on plasma protein leakage and role of sensory neurons. *J Appl Physiol.* **72**(4):1418-24.
- RAJAKULASINGAM K, POLOSA R, LAU LC, CHURCH MK, HOLGATE ST, HOWARTH PH (1993). Comparative nasal effects of bradykinin and histamine: influence on nasal airways resistance and plasma protein exudation. *Thorax.* **48**(4):324-9.
- RAMIS I, SERRA J, ROSELLO J, PICADO C, GELPI E, VIDAL AA (1988). PGE<sub>2</sub> and PGF<sub>2</sub> alpha in the nasal lavage fluid from healthy subjects: methodological aspects. *Prostaglandins Leukot Essent Fatty Acids.* **34**(2):109-12.
- RAMSAY SG, CLAYTON RA, DAGG KD, THOMSON LJ, NALLY JE, THOMSON NC (1997). Effect of angiotensin II on histamine-induced bronchoconstriction in the human airway both in vitro and in vivo. *Respir Med.* **91**(10):609-15.
- RANGI SP, SAMPLE S, SERWONSKA MH, LENAHA GA, GOETZL EJ (1990a). Mediation of prolonged increases in nasal mucosal blood flow by calcitonin gene-related peptide (CGRP). *J Clin Immunol.* **10**(6):304-10.
- RANGI SP, SERWONSKA MH, LENAHA GA, PICKETT WC, BLAKE VA, SAMPLE S, GOETZL EJ (1990b). Suppression by ingested eicosapentaenoic acid of the increases in nasal mucosal blood flow and eosinophilia of ryegrass-allergic reactions. *J Allergy Clin Immunol.* **85**(2):484-9.
- RAPHAEL GD, IGARASHI Y, WHITE MV, KALINER MA (1991). The pathophysiology of rhinitis. V. Sources of protein in allergen-induced nasal secretions. *J Allergy Clin Immunol.* **88**(1):33-42.
- RAPHAEL GD, MEREDITH SD, BARANIUK JN, DRUCE HM, BANKS SM, KALINER MA (1989). The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis.* **139**(3):791-800.
- REPKA-RAMIREZ MS (2003). New concepts of histamine receptors and actions. *Curr Allergy Asthma Rep.* **3**(3):227-31.
- RIEDERER A, FISCHER A, KNIPPING S, UNGER J, LANGE W, KASTENBAUER E (1996). Basic innervation pattern and distribution of classic autonomic neurotransmitters in human nasal mucosal vasculature. *Laryngoscope.* **106**(3 Pt 1):286-91.
- RILEY JF, WEST GB (1952). Histamine in tissue mast cells. *J Physiol.* **117**(4):72P-73P.
- SAITO H, MATSUMOTO K, DENBURG AE, CRAWFORD L, ELLIS R, INMAN MD, SEHMI R, TAKATSU K, MATTHAEI KI, DENBURG JA (2002). Pathogenesis of Murine Experimental Allergic Rhinitis: A Study of Local and Systemic Consequences of IL-5 Deficiency. *J Immunol.* **168**(6): 3017-23.
- SAKURAI E, GUNJI E, IIZUKA Y, HIKICHI N, MAEYAMA K, WATANABE T (1994). The disposition of thioperamide, a histamine H<sub>3</sub>-receptor antagonist, in rats. *J Pharm Pharmacol.* **46**(3):209-12.
- SAKURAI E, SAKURAI E, TANAKA Y, WATANABE T, SINGH JOSSAN S, ORELAND L (2001). Effects of histamine H<sub>3</sub>-receptor ligands on brain monoamine oxidase in various mammalian species. *Brain Res.* **906**(1-2):180-3.
- SAMPSON AP (2000). The role of eosinophils and neutrophils in inflammation. *Clin Exp Allergy.* **30 Suppl 1**:22-7.

- SANICO AM, ATSUTA S, PROUD D, TOGIAS A (1997). Dose-dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. *J Allergy Clin Immunol.* **100**(5):632-41.
- SASAKI Y, IHARA K, AHMED S, YAMAWAKI K, KUSUHARA K, NAKAYAMA H, NISHIMA S, HARA T (2000). Lack of association between atopic asthma and polymorphisms of the histamine H<sub>1</sub> receptor, histamine H<sub>2</sub> receptor, and histamine N-methyltransferase genes. *Immunogenetics.* **51**(3):238-40.
- SAXENA PR (1975). The significance of histamine H<sub>1</sub> and H<sub>2</sub> receptors on the carotid vascular bed in the dog. *Neurology.* **25**(7):681-7.
- SHELLENBERG RR, DUFF MJ, FOSTER A, PADDON HB (1986). Histamine releases PGI<sub>2</sub> from human pulmonary artery. *Prostaglandins.* **32**(2):201-9.
- SCHENKEL E, CORREN J, MURRAY JJ (2002). Efficacy of once-daily desloratadine/pseudoephedrine for relief of nasal congestion. *Allergy Asthma Proc.* **23**(5):325-30.
- SCHIERHORN K, BRUNNEE T, SCHULTZ KD, JAHNKE V, KUNKEL G (1995). Substance-P-induced histamine release from human nasal mucosa in vitro. *Int Arch Allergy Immunol.* **107**(1-3):109-14.
- SCHIMMER BP, PARKER KL (1996). ACTH; Adrenocortical steroids and their synthetic analogs. *Goodman & Gilman's The pharmacological basis of therapeutics 9th edition.* Molinoff and Ruddon (eds). Chapter **59**:1459-85.
- SCHNEIDER E, ROLLI-DERKINDEREN M, AROCK M, DY M (2002). Trends in histamine research: new functions during immune responses and hematopoiesis. *Trends Immunol.* **23**(5):255-63.
- SCHULMAN ES, POST TJ, HENSON PM, GICLAS PC (1988). Differential effects of the complement peptides, C5a and C5a des Arg on human basophil and lung mast cell histamine release. *J Clin Invest.* **81**(3):918-23.
- SECHER C, KIRKEGAARD J, BORUM P, MAANSSON A, OSTERHAMMEL P, MYGIND N (1982). Significance of H<sub>1</sub> and H<sub>2</sub> receptors in the human nose: rationale for topical use of combined antihistamine preparations. *J Allergy Clin Immunol.* **70**(3):211-8.
- SERLE JB, PODOS SM, LUSTGARTEN JS, TEITELBAUM C, SEVERIN CH (1985). The effect of corynanthine on intraocular pressure in clinical trials. *Ophthalmology.* **92**(7):977-80.
- SHELTON D, EISER N (1994). Histamine receptors in the human nose. *Clin Otolaryngol.* **19**(1):45-9.
- SHIMOKAWA H, YASUTAKE H, FUJII K, OWADA MK, NAKAIKE R, FUKUMOTO Y, TAKAYANAGI T, NAGAO T, EGASHIRA K, FUJISHIMA M, TAKESHITA A (1996). The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol.* **28**(5):703-11.
- SHIRASAKI H, KANAIZUMI E, WATANABE K, MATSUI T, SATO J, NARITA S, RAUTIAINEN M, HIMI T (2002). Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa. *Clin Exp Allergy.* **32**(7):1007-12.
- SILVER RB, POONWASI KS, SEYEDI N, WILSON SJ, LOVENBERG TW, LEVI R (2002). Decreased intracellular calcium mediates the histamine H<sub>3</sub>-receptor-induced attenuation of

norepinephrine exocytosis from cardiac sympathetic nerve endings. *Proc Natl Acad Sci USA*. **99**(1):501-6.

SIM TC, REECE LM, HILSMEIER KA, GRANT JA, ALAM R (1995). Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med*. **152**(3):927-33.

SIMONS FE, GRIMM PG (1996). Histamine H<sub>1</sub>-receptors in the nasal mucosa: a mystery solved? *Clin Exp Allergy*. **26**(4):368-70.

SIMONS FE, PRENNER BM, FINN A JR (2003). Efficacy and safety of desloratadine in the treatment of perennial allergic rhinitis. *J Allergy Clin Immunol*. **111**(3):617-22.

SIRAGANIAN RP (2003). Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol*. **15**(6):639-46.

SIROIS J, MENARD G, MOSES AS, BISSONNETTE EY (2000). Importance of histamine in the cytokine network in the lung through H<sub>2</sub> and H<sub>3</sub> receptors: stimulation of IL-10 production. *J Immunol*. **164**(6):2964-70.

SMAILBEGOVIC A, LEVER R, PAGE CP (2001). The effects of heparin on the adhesion of human peripheral blood mononuclear cells to human stimulated umbilical vein endothelial cells. *Br J Pharmacol*. **134**:827-36.

STEWART GA, THOMPSON PJ, MCWILLIAM AS (1993). Biochemical properties of aeroallergens: contributory factors in allergic sensitization? *Pediatr Allergy Immunol*. **4**(4):163-72.

STJARNE P (1991). Sensory and motor reflex control of nasal mucosal blood flow and secretion; clinical implications in non-allergic nasal hyperreactivity. *Acta Physiol Scand Suppl*. **600**:1-64.

STUMP DC, MACFARLANE DE (1983). Clonidine and para-aminoclonidine, partial agonists for the alpha<sub>2</sub>-adrenergic receptor on intact human blood platelets. *J Lab Clin Med*. **102**(5):779-87.

SUR S, GLITZ DG, KITA H, KUJAWA SM, PETERSON EA, WEILER DA, KEPHART GM, WAGNER JM, GEORGE TJ, GLEICH GJ, LEIFERMAN KM (1998). Localization of eosinophil-derived neurotoxin and eosinophil cationic protein in neutrophilic leukocytes. *J Leukoc Biol*. **63**(6):715-22.

SVENSSON C, BAUMGARTEN CR, PIPKORN U, ALKNER U, PERSSON CG (1989). Reversibility and reproducibility of histamine induced plasma leakage in nasal airways. *Thorax*. **44**(1):13-8.

SVENSSON G, HEGARDT B, LOFKVIST T (1980). Effects of topical use of beta-adrenoceptor stimulants of nasal mucosa. Rhinomanometric evaluations in experiments with terbutaline and KWD 2131. *Acta Otolaryngol*. **90**(3-4):297-303.

TAKENO S, KAWAMOTO H, HIRATA S, FUKUSHIMA N, YAZIN K (1998). Decongesting effect of tramazoline on nasal airway patency and nasal symptoms as evaluated by acoustic rhinometry: an objective study in 30 allergic and six non-allergic subjects. *Nippon Jibiinkoka Gakkai Kaiho*. **101**(7):900-7.

TANAKA T, STARKE K (1980). Antagonist/agonist-preferring alpha-adrenoceptors or alpha<sub>1</sub>/alpha<sub>2</sub>-adrenoceptors? *Eur J Pharmacol*. **63**(2-3):191-4.

- TARDIVEL-LACOMBE J, MORISSET S, GBAHOU F, SCHWARTZ JC, ARRANG JM (2001). Chromosomal mapping and organization of the human histamine H<sub>3</sub> receptor gene. *Neuroreport*. **12**(2):321-4.
- TASMAN AJ, BOGATZKI B, HEPPT W, HAUSER-KRONBERGER C, FISCHER A (1998). Nitric oxide synthase in the innervation of the human nasal mucosa: correlation with neuropeptides and tyrosine hydroxylase. *Laryngoscope*. **108**(1 Pt 1):128-33.
- THAETE LG, SPICER SS, SPOCK A (1981). Histology, ultrastructure, and carbohydrate cytochemistry of surface and glandular epithelium of human nasal mucosa. *Am J Anat*. **162**(3):243-63.
- TODA N, OKAMURA T (1989). Endothelium-dependent and -independent responses to vasoactive substances of isolated human coronary arteries. *Am J Physiol*. **257**(3 Pt 2):H988-95.
- TOGIAS A (2003). H<sub>1</sub>-receptors: localization and role in airway physiology and in immune functions. *J Allergy Clin Immunol*. **112**(4 Suppl):S60-8.
- TOGIAS A, NACLERIO RM, PROUD D, PIPKORN U, BASCOM R, ILIOPOULOS O, KAGEY-SOBOTKA A, NORMAN PS, LICHTENSTEIN LM (1988). Studies on the allergic and nonallergic nasal inflammation. *J Allergy Clin Immunol*. **81**(5 Pt 1):782-90.
- TRENDELENBURG U (1984). The influence of inhibition of catechol-O-methyl transferase or of monoamine oxidase on the extraneuronal metabolism of 3H(-)-noradrenaline in the rat heart. *Naunyn Schmiedebergs Arch Pharmacol*. **327**(4):285-92.
- TURNER PJ (1999). Mechanisms of hyperresponsiveness in the human nasal airway: Role of kinins and nitric oxide. *UCL PhD Thesis*.
- TURNER PJ, DEAR JW, FOREMAN JC (2000a). Involvement of kinins in hyperresponsiveness induced by platelet activating factor in the human nasal airway. *Br J Pharmacol*. **129**(3):525-32.
- TURNER PJ, FOREMAN JC (1999). Hyperresponsiveness in the human nasal airway: new targets for the treatment of allergic airway disease. *Mediators Inflamm*. **8**(3):133-46.
- TURNER PJ, MAGGS JR, FOREMAN JC (2000b). Induction by inhibitors of nitric oxide synthase of hyperresponsiveness in the human nasal airway. *Br J Pharmacol*. **131**(2):363-9.
- TURNER P, DEAR J, SCADDING G, FOREMAN JC (2001). Role of kinins in seasonal allergic rhinitis: icatibant, a bradykinin B<sub>2</sub> receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen. *J Allergy Clin Immunol*. **107**(1):105-13.
- TYRRELL DJ, HORNE AP, HOLME KR, PREUSS JM, PAGE CP (1999). Heparin in inflammation: potential therapeutic applications beyond anticoagulation. *Adv Pharmacol*. **46**:151-208.
- TYRRELL DJ, KILFEATHER S, PAGE CP (1995). Therapeutic uses of heparin beyond its traditional role as an anticoagulant. *Trends in Pharmacol Sci*. **16**(6):198-204.
- U'PRICHARD DC, GREENBERG DA, SNYDER SH (1977). Binding characteristics of a radiolabeled agonist and antagonist at central nervous system alpha noradrenergic receptors. *Mol Pharmacol*. **13**(3):454-73.



- URIEN S, TILLEMENT JP, GANEM B, KUCH MD (1999). A pharmacokinetic-pharmacodynamic modelling of the antihistaminic ( $H_1$ ) effects of cetirizine. *Int J Clin Pharmacol Ther.* **37**(10):499-502.
- VALENTINE AF, RIZZO CA, RIVELLI MA, HEY JA (1999). Pharmacological characterization of histamine  $H_3$  receptors in human saphenous vein and guinea pig ileum. *Eur J Pharmacol.* **366**(1):73-8.
- VAN AEDELSBERG J, PHILIP G, PEDINOFF AJ, MELTZER EO, RATNER PH, MENTEN J, REISS TF (2003). Montelukast improves symptoms of seasonal allergic rhinitis over a 4-week treatment period. *Allergy.* **58**(12):1268-76.
- VAN CAUWENBERGE PB (1997). Nasal sensitization. *Allergy.* **52**(33 Suppl):7-9.
- VAN DER GOOT H, TIMMERMAN H (2000). Selective ligands as tools to study histamine receptors. *Eur J Med Chem.* **35**(1):5-20.
- VAN EULER US (1972). Synthesis, uptake and storage of catecholamines in adrenergic nerves, the effect of drugs. *Handb Exp Pharm.* **33**(Chapter 6):186-230.
- VAN HECKEN AM, TJANDRAMAGA TB, MULLIE A, VERBESSELT R, DE SCHEPPER PJ (1982). Ranitidine: single dose pharmacokinetics and absolute bioavailability in man. *Br J Clin Pharmacol.* **14**(2):195-200.
- VAN MEGEN YJ, KLAASSEN AB, RODRIGUES DE, MIRANDA JF, VAN GINNEKEN CA, WENTGES BT (1991). Alterations of adrenoceptors in the nasal mucosa of allergic patients in comparison with nonallergic individuals. *J Allergy Clin Immunol.* **87**(2):530-40.
- VAN WIJK RG, DE GRAAF-IN 'T VC, GARRELDs IM (1999). Nasal hyperreactivity. *Rhinology.* **37**(2):50-5.
- VAN WIJK RG, ZIJLSTRA FJ, VAN TOORENENBERGEN AW, VERMEULEN A, DIEGES PH (1992). Isolated early response after nasal allergen challenge is sufficient to induce nasal hyperreactivity. *Ann Allergy.* **69**(1):43-7.
- VANCHERI C, MASTRUZZO C, ARMATO F, TOMASELLI V, MAGRI S, PISTORIO MP, LAMICELA M, D'AMICO L, CRIMI N (2001). Intranasal heparin reduces eosinophil recruitment after nasal allergen challenge in patients with allergic rhinitis. *J Allergy Clin Immunol.* **108**(5 Part 1):703-8.
- VARNEY V (1991). Hayfever in the United Kingdom. *Clin Exp Allergy.* **21**(6):757-62.
- VARTY L, HEY J (2002). Histamine  $H_3$  receptor activation inhibits neurogenic sympathetic vasoconstriction in porcine nasal mucosa. *Eur J Pharmacol.* **452**(3):339.
- VARTY LM, GUSTAFSON E, LAVERTY M, HEY JA (2004). Activation of histamine  $H_3$  receptors in human nasal mucosa inhibits sympathetic vasoconstriction. *Eur J Pharmacol.* **484**(1):83-9.
- WALDEN SM, PROUD D, BASCOM R, LICHTENSTEIN LM, KAGEY-SOBOTKA A, ADKINSON NF Jr., NACLERIO RM (1988). Experimentally induced nasal allergic responses. *J Allergy Clin Immunol.* **81**(5 Pt 2):940-9.
- WALSH MP (1994). Regulation of vascular smooth muscle tone. *Can J Physiol Pharmacol.* **72**(8):919-36.

- WANG D, CLEMENT P, SMITZ J (1996). Effect of H<sub>1</sub> and H<sub>2</sub> antagonists on nasal symptoms and mediator release in atopic patients after nasal allergen challenge during the pollen season. *Acta Otolaryngol.* **116**(1):91-6.
- WANG DY, HANOTTE F, DE VOS C, CLEMENT P (2001). Effect of cetirizine, levocetirizine, and dextrocetirizine on histamine-induced nasal response in healthy adult volunteers. *Allergy.* **56**(4):339-43.
- WANG HW, JACKSON RT (1988). Do cholinergic neurons directly innervate nasal blood vessels? *Rhinology.* **26**(2):139-46.
- WANG M, LUNG MA (2003). Adrenergic mechanisms in canine nasal venous systems. *Br J Pharmacol.* **138**(1):145-55.
- WANG SH, WANG HW, WANG JY (1993). Effects of cocaine on human nasal mucosa. *Eur Arch Otorhinolaryngol.* **250**(4):245-8.
- WHITTET HB, FISHER EW (1988). Nasal obstruction after cervical sympathectomy: Homer's syndrome revisited. *J Otorhinolaryngol Relat Spec.* **50**(4):246-50.
- WIDDICOMBE J (1997). Microvascular anatomy of the nose. *Allergy.* **52**(40 Suppl):7-11.
- WIDDICOMBE JG (1990). Nasal pathophysiology. *Respir Med.* **84** Suppl A:3-9; discussion 9-10.
- WIGHT RG, COCHRANE T (1989). A comparison of the effects of xylometazoline on nasal airflow, and on blood flux as measured by laser Doppler flowmetry. *Acta Otolaryngol.* **108**(3-4):284-9.
- WILLEMSSEN JJ, ROSS HA, WOLTERS BG, SWEEP CG, KEMA IP (2001). Evaluation of specific high-performance liquid-chromatographic determinations of urinary adrenaline and noradrenaline by comparison with isotope dilution mass spectrometry. *Ann Clin Biochem.* **38**(Pt 4):356-64.
- WILSON AM, HAGGART K, SIMS EJ, LIPWORTH BJ (2002). Effects of fexofenadine and desloratadine on subjective and objective measures of nasal congestion in seasonal allergic rhinitis. *Clin Exp Allergy.* **32**(10):1504-9.
- WOOD-BAKER R, LAU L, HOWARTH PH (1996). Histamine and the nasal vasculature: the influence of H<sub>1</sub> and H<sub>2</sub>-histamine receptor antagonism. *Clin Otolaryngol.* **21**(4):348-52.
- WORTH AP, CRONIN MT (2001). The use of pH measurements to predict the potential of chemicals to cause acute dermal and ocular toxicity. *Toxicology.* **169**(2):119-31.
- XIONG Y, KARUPIAH G, HOGAN SP, FOSTER PS, RAMSAY AJ (1999). Inhibition of allergic airway inflammation in mice lacking nitric oxide synthase 2. *J Immunol.* **162**(1):445-52.
- YAMASAKI T, TAMAI I, MATSUMURA Y (2001). Activation of histamine H<sub>3</sub> receptors inhibits renal noradrenergic neurotransmission in anesthetized dogs. *Am J Physiol Regul Integr Comp Physiol.* **280**(5):R1450-6.
- YANG P, LI X (1995). Induction of endogenous anti-IgE antibody of nasal mucosa and its inhibitive effects on IgE level. *Zhonghua Er Bi Yan Hou Ke Za Zhi.* **30**(1):27-9.

YOUSIF MH, ORIOWO MA, CHERIAN A, ADEAGBO AS (2002). Histamine-induced vasodilatation in the perfused mesenteric arterial bed of diabetic rats. *Vascul Pharmacol.* **39**(6):287-92.

ZANOLETTI E, DE PAOLI F, REGAZZI BONORA M, CASTENUOVO P, MEVIO E, MIRA E (1999). Modification in some nasal function parameters after metacholine stimulation and pretreatment with ipratropium bromide. *Acta Otorhinolaryngol Ital.* **19**(3):150-4.

ZHAO QE, MIHARA T, SUGIMOTO Y, KAMEI C (1996). Mechanism of Bradykinin-induced histamine release from rat peritoneal mast cells. *Biol Pharm Bull.* **19**(2):237-40.

ZHU Y, MICHALOVICH D, WU H, TAN KB, DYTCKO GM, MANNAN IJ, BOYCE R, ALSTON J, TIERNEY LA, LI X, HERRITY NC, VAWTER L, SARAU HM, AMES RS, DAVENPORT CM, HIEBLE JP, WILSON S, BERGSMA DJ, FITZGERALD LR (2001). Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol.* **59**(3):434-41.

ZWEIMAN B, GETSY J, KALENIAN M, LANE A, SCHWARTZ LB, DOTY R, LANZA D (1997). Nasal airway changes assessed by acoustic rhinometry and mediator release during immediate and late reactions to allergen challenge. *J Allergy Clin Immunol.* **100**(5):624-31.